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ELASTOMERS FOR FUEL SYSTEMS CONTAINING MICROORGANISM-CONTROLLING ADDITIVES

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FOREWORD

This report was prepared by Battelle Memorial Institute under RTD Contract No. AF 33(657)-9804, Project No. 7340, "Nonmetallic and Composite Materials", Task No. 734005, "Elastomeric and Compliant Materials", and Project No. 7381, "Materials Applications", Task No. 738101, "Exploratory Design and Prototype Development". Administration is under the Applications Division, AF Materials Laboratory, Research and Technology Division, Wright-Patterson Air Force Base, with Mr. Philip A. House acting as Project Engineer.

This final report summarizes work done during the period from October 1, 1962, through September 30, 1964. The manuscript was released by the authors on January 31, 1965 for publication as an RTD Technical Report.

This report has been reviewed and is approved.

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ABSTRACT

This program was part of a major study by the Air Force to overcome problems created by the undesirable growth of microorganisms in aircraft jet-fuel tanks and ground storage tanks. Problems attributed to microbial growth include plugging of fuel gages, destruction of topcoatings, and corrosion of metal parts.

It was shown that certain species of bacteria and fungi, isolated from JP-4 fuel, grow rapidly on coatings in laboratory exposures. Damage to coatings after 1 year of exposure was minimal, suggesting that growth alone is not as important as growth augmented by other factors such as corrosive materials in fuel-tank water bottoms. For example, aircraft fuel sump samples (without microorganisms) from various USAF bases were shown to be corrosive to both uncoated and coated 7075-T6 aluminum and to cause blistering of a topcoating.

Although coating damage and metal corrosion could not be attributed to microbial action alone, control of microbial growth in fuel tanks is still desirable. Several biocides were found which inhibit growth when added to coatings. Two of these in particular were effective at relatively low concentrations and are recommended for further study. It was also shown that three biocides selected for addition to fuel are not harmful to most existing fuel-system coatings and elastomeric components, although one caused embrittlement of a nitrile rubber specimen.

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ELASTOMERS FOR FUEL SYSTEMS CONTAINING MICROORGANISM-CONTROLLING ADDITIVES

by

C. W. Cooper, H. T. Kemp, and R. M. Kell

INTRODUCTION

This is a summary report covering activity during the 2-year period from October 1, 1962, through September 30, 1964, on Contract No. AF 33(657)-9804, Task Numbers 734005 and 738101. The study initially concerned research on means for improving the resistance to microbial deterioration of jet-fuel system coatings and sealants used in aircraft. This was expanded on November 1, 1963, to include coatings for steel ground-storage tanks and carriers. The program also included an investigation of the possible effect on aircraft fuel system sealants, coatings and elastomers of biocide additives for fuel developed in parallel programs conducted by other contractors.

One of the major problems of the Air Force has been the existence of microorganisms in fuel systems. These microorganisms were believed responsible for severe corrosion damage to metal surfaces. Work has been conducted along several fronts by the Air Force and by a number of contractors to alleviate this situation. The various approaches include (1) the investigation of new protective coating materials, (2) the addition of biocidal agents to fuel, (3) a similar addition of biocides to selected coating materials, and (4) improvement in housekeeping, fuel handling, and filtration. The Battelle program has been concerned with the development of jet-fuel systems coatings and sealants with resistance to microbial growth and deterioration along the lines indicated in Item (3).

Primary objectives of the study were as follows:

(1) Select most active microbial isolates for coating exposure and adapt cultures to specific environment, i.e., fuel, water, coatings, and sealants.

(2) Determine the effect of selected microorganisms on a variety of coating materials.

(3) Select and evaluate a large number of biocides as potentially effective growth-controlling additives to selected coatings for aluminum and steel.

(4) Determine the effect of selected fuel additive (furnished by RTD) on the properties of various coatings, sealants, and elastomeric components of fuel systems.

One corollary but rather important aspect of the program has been the determination of the corrosive nature of samples obtained from fuel sumps of aircraft at various strategic locations.

During the course of this program, other effective methods of controlling g[illegible] and minimizing attendant difficulties have been brought to light by the Air Force. This has occurred as a result of a better understanding of the total problem. Such approaches as better housekeeping and improved fuel handling, storage, and filtration have been used quite successfully. One of the main reasons for a reduction in concern regarding microbial growth has been the biocidal effect of the ethylene glycol monomethyl ether component of the anti-icing additive now used in jet fuels. In spite of this, aluminum corrosion remains as a possible serious difficulty in fuel sumps and low spots of tanks of idle aircraft. Thus, adequate protection of metal surfaces by means of effective coatings is still of prime importance.

SUMMARY AND CONCLUSIONS

Commercial coating materials to be evaluated in the program originally outlined were those used or considered for use on anodized or Iridited treated 7075-T6 aluminum. In November, 1963, the study was broadened at the request of the Research and Technology Division to include coatings for steel (specification QQ-S-636) used in ground storage tanks and fuel carriers. A total of 26 coatings and sealants were exposed to an environment designed to promote growth of selected microorganisms. These coatings and sealants included materials representative of the following classes: Buna N, one- and two-part polyurethane, polysulfide, nylon, furan, inorganic zinc, epoxy-polysulfide, epoxy, fluoropolymer, silicone, and fluorinated silicone.

One hundred twenty-seven cultures of microbial isolates from jet fuel were received from various sources. Of these, 34 grew well in a simulated aircraft fuel-tank environment consisting of JP-4 fuel, water, and an aluminum adaptation strip covered with representative coating and sealant materials. The five most active microorganisms were selected for use in mixed inoculum experiments used throughout the research program. These were three pseudomonad bacteria and two fungus species of the Hormodendron-Cladosporium group.

Growth ultimately appeared on all coatings not protected by biocidal additives. This usually occurred within 7 days of exposure to the mixed microbial system. Certain materials, however, apparently contained inhibitory components which delayed profuse growth. A furan-type and an epoxy/polysulfide coating retarded growth attachment for up to 28 days, a dichromate-cured polysulfide for 35 days, and an epoxy for up to 56 days. In spite of the attachment of growth, there was little visual evidence of coating damage after long-term exposure to fuel and water mixtures containing microorganisms.

Various means were investigated for evaluating coatings as a result of the difficulty encountered in detecting damage or change resulting from microbial exposure. These methods included a pencil-hardness test, light microscopy, electron microscopy, penetration tests, moisture-vapor transmission measurements, a dye technique, an electrographic method, and measurement of electrolytic resistance. The latter appeared to offer the greatest promise, and in some instances provided the only means of noting a deleterious effect.

The use of biocidal additives was investigated as a means of preventing attached growth and possible attendant deteriorative effects on coatings. A two-component

polyurethane coating for aluminum and an epoxy coating for steel were specified by RTD for this study. Biocidal additives were solicited from a variety of sources, and 92 such materials were screened. Certain of the more common commercial products of this nature were unsatisfactory for this purpose because of general requirements for low fuel and water solubility plus the necessity for avoiding heavy metals, halogens, or sulfur thought to be corrosive or damaging to aluminum or to engine parts.

The study has also been concerned with an investigation of the possible adverse effect of biocidal additives for fuel on elastomers, bladder cell materials, repair cements, and coatings used in aircraft fuel systems. The fuel biocides were developed in programs conducted elsewhere. With one exception, the three biocide additives submitted to Battelle by RTD were found to be compatible with the fuel-system components. The exception involved an apparent embrittlement of a nitrile rubber compound in the presence of a biocide identified as arsenosobenzene.

A number of important conclusions have been reached as a result of this research program and the following points are emphasized:

(1) Attached growth appeared on all coatings or sealants not protected by biocidal additives. This occurred usually within a 7-day period, although certain products inhibited growth for periods up to 56 days.

(2) In microbial exposures of 1 year, no gross deterioration has been noted in any of the coatings or sealants investigated.

(3) There is some evidence of minor change in coatings on continued exposure to microorganisms, as shown by a sensitive method for measuring the electrolytic resistance.

(4) Most physical properties of a two-component polyurethane coating are not apparently affected by the addition of up to 20 parts of a selected biocide. The biocide-containing coating passed major qualification tests of MIL-C-27725A, such as fuel and water resistance and low-temperature flexibility.

(5) One biocide has been found which has performed well at concentrations as low as 5 parts per 100 parts of polyurethane coating. A second biocide was found which provided protection at a 10-part level.

(6) The most effective biocide, an organic thiocyanate, prevented growth for 240 days on both a two-part polyurethane and an epoxy coating in the presence of the mixed microbial inoculum. When the exposure medium of fuel, water, and microorganisms was replaced every tenth day, this biocide was continuing to provide protection for the coating after 40 days.

(7) There is strong evidence that the pH of a fuel-water mixture becomes progressively lower as microbial growth increases. This confirms the suspected relationship between growth and corrosion and indicates a need for effective surface coatings.

(8) All water bottoms collected from SAC bases were corrosive to untreated, anodized, or Iridite-treated 7075-T6 aluminum. On exposure of Buna N and polyurethane-coated aluminum specimens to these water bottoms, blisters occurred in the case of the Buna N material while the polyurethane coating was not affected visibly.

RECOMMENDATIONS

At the outset of this study it was believed that microorganisms were playing an active role in deterioration of coatings for integral jet-fuel tanks and in metal corrosion subsequent to this. During the course of the work, however, thinking changed somewhat. First, it has not been shown conclusively in laboratory studies that gross degradation of coatings occurs as a result of exposure to typical microorganisms. Second, as mentioned elsewhere in this report, certain other approaches have been used quite effectively to control growth in fuel systems. However, the presence of microbial growth in fuel systems is certainly undesirable, and further means for its control would be of considerable advantage especially under extreme circumstances such as during prolonged military action or in tropical areas. Thus, it will be desirable to take advantage of knowledge gained as a result of this program and other related ones. In order to realize the greatest benefit from this work, the following recommendations are made:

(1) Certain established biocides which have been used on an industrial scale for many years have not been effective as microorganism-controlling additives for fuel-tank coatings. On the other hand, certain other additives not well known as biocides have performed well. The reasons for this are not understood and should be investigated.

(2) Continue environmental exposures of preferred biocides, with special emphasis on those materials identified as AC-1 and AC-3. These (particularly the latter material, which was received late in the program) should be more thoroughly tested to obtain performance data at minimum effective levels.

(3) Evaluation of selected coatings with and without biocides should be carried out in fuel-water mixtures containing known corrosive ions or agents found in fuel samples. This should be done in the presence and absence of microorganisms selected in this study. Results of such a test will supply additional evidence needed to confirm or disprove the active role of microbial action in corrosion.

(4) One of the more important conclusions reached in this work has been the fact that coatings are needed with improved resistance to water, better adhesion to metal surfaces under adverse conditions, and greater ease of handling. A single-component material would be of great advantage. Preferably, such a coating should be inherently biocidal – at least to the extent that growth would not attach to it. It is recommended that developments along this line be considered. From knowledge presently at hand, it is believed that certain more conventional coating materials not presently used in aircraft might be adapted advantageously for this purpose. An example would be the tung oil phenolic varnishes.

EXPERIMENTAL WORK

Coatings and Sealants Evaluated

At the beginning of this study, the jet-fuel problem was especially apparent in integral wing-tank sections of aircraft. Consequently, initial studies were made with sealants and coatings commonly used in wing tanks of Air Force planes. These included polysulfide sealants, a Buna N coating, and a two-part polyurethane topcoating. Later, other coating materials were added to the study to cover as many representative materials as possible. Such diverse types of coatings as silicones, nylons, one-part polyurethanes, epoxies, and others were included. Several of these were not intended by their manufacturer as wing-tank coatings. Nevertheless, it was felt desirable to include them in the study to have as much information as possible on the microbial resistance of various types of coatings. In addition, part way through the program it was decided to extend the study of microbial resistance to coatings typical of those used on the interior of steel ground-storage tanks and fuel carriers. Consequently, six coatings of this type were added to the study. This gave a total of 26 coatings that were evaluated for resistance to microorganisms. Coatings and sealants used in the study have been given a code letter designation throughout this report. A list of these materials is given in Table 1, which also includes the chemical type and intended service for the material.

TABLE 1. COATINGS AND SEALANTS FOR ALUMINUM AND STEEL

Code Letter[a]	Type	Code Letter[a]	Type
A	Buna N	Q	Epoxy/polysulfide
B	Two-part polyurethane	R	Polyurethane
C	Polysulfide	S	Fluoropolymer
D	Polysulfide	T	Fluorinated silicone
E	One-part polyurethane	U	Epoxy
F	One-part polyurethane	V	Epoxy
G	One part polyurethane	W	Epoxy
H	Nylon	X	Silicone
I	Furan	Y	Fluoropoly.
J	Furan	Z	Fluoropolymer
K	One-part polyurethane		
L	Inorganic zinc	S-1	Polysulfide, manganese cure
M	Epoxy	S-2	Polysulfide, dichromate cure
N	Epoxy	S-3	Polysulfide, manganese cure
O	Epoxy	S-4	Polysulfide, lead cure
P	Epoxy		

(a) A-Z are coatings; S-1 — S-4 are sealants.

Polysulfide sealants were not included in the mixed microbial exposures because the Air Force reportedly has never experienced microbial degradation of these materials. However, two coatings, similar in composition to sealants but thinned to permit their use as coatings, were included in the study. Thus, information on the microbial resistance of polysulfides has been obtained. In addition, the polysulfide sealants were used in other portions of the study dealing with: (1) the effect of the addition of biocides to sealants and coatings, and (2) the effect of incorporation of a biocide in the fuel on the physical properties of sealants and other fuel-system elastomers. These other elastomeric materials are listed in the section on biocides under the heading Effect on Physical Properties of Coating.

All coatings used in microbial studies were applied to aluminum specimens by a fill-and-drain method. Aluminum specimens consisted initially of 1 x 5-inch panels of 0.050-inch 7075 T6 aluminum. It was found later that coating failure most often started at the edge of these panels. To avoid this edge effect and thus improve reproducibility of test results, subsequent specimens were prepared using 3/8-inch aluminum rods 5 inches long, radius rounded at one end. Before applying coatings, aluminum specimens were cleaned according to the method described in Paragraph 4.6.2.2 of MIL-C-27725 USAF. This consists essentially of a solvent wipe, using a mixture of ester, ketone, alcohol, and hydrocarbon solvents. Both Iridited and anodized rods were used in the study.

A fill-and-drain method of applying the coatings to the aluminum specimens was selected because it gave a more uniform coating than one applied by brush and fewer air bubbles than occurred with air-sprayed coatings. The fill-and-drain procedure is used in applying coatings to some Air Force planes. The laboratory apparatus consisted of a glass container holding two aluminum specimens suspended vertically. The container was filled with a coating and then slowly drained through a valve in the bottom. All coatings were air-dried at room temperature, according to directions from the manufacturer. In most cases this amounted to a drying period of 7 days. However, a two-part polyurethane (Coating B) required a 14-day cure.

Polysulfide sealants were used in some studies involving biocide compatibility and certain other tests described in this report. These sealants were applied to both anodized and Iridited aluminum panels, the size of the panel being specified in MIL-S-8802C or MIL-C-27725A. All test panels were cleaned using the solvent described above. Sealants were mixed in a Semco Model 1350 mixer, and placed in cartridges for extrusion on test panels using a Semco No. 250 gun. In some cases, coated panels were placed in Teflon-coated molds to insure uniform thickness of the cured sealant. Sealants were air-cured at room temperature for 14 days before testing.

Microbiological Studies

Acquisition, Culture, and Adaptation of Jet-Fuel Isolates

One hundred twenty-seven cultures of jet-fuel isolates were received from various organizations. Identifications of the microorganisms, their source, and their degree of growth upon modified Bushnell-Haas (B-H) agar medium slants overlaid with Searsport* JP-4 fuel (system described below) are presented in Table 2.

A majority of the microorganisms were identified by previous workers at least as to genus, and a number were identified as to genus and species. With the exception of B-55 and B-62 through B-65, all appear to be pure cultures. All isolates received in jet-fuel culture grew well in the jet-fuel culture system described below. However, difficulty in obtaining good growth was experienced with those received on standard laboratory media. Only two isolates (B-66 and B-67) grew well in fuel on the first attempt when transferred from standard laboratory media.

The initial transfers were made from original cultures into a B-H slant jet-fuel-culture system. Prescription bottles (8-oz) were selected as the most suitable for this

*JP-4 fuel from Searsport, Maine, was used throughout the program.

TABLE 2. IDENTIFICATION, SOURCE, AND GROWTH IN FUEL OF MICROORGANISMS MAINTAINED IN STOCK CULTURE FOR USE IN JET FUEL-ELASTOMER STUDIES

Battelle Code No.[a]	Other Code No.[b]	Identification	Other Information	Growth in Fuel Culture System at 30 C, 14 days[c]
B-1	197-4	Bacillus cereus		+
B-2	513-4	B. cereus (var.)		+
B-3	099-12	B. megatherium		+
B-4	030-Hy4b	B. megatherium (var.)		+
B-5	189-7	Azotomonas insolita		+
B-6	2606-25	Micrococcus sp.		+
B-7	2606-24	Staphylococcus epidermitis		+
B-8	2606-36	Staphylococcus epidermitis (var.)		+
B-9	706-9	Pseudomonas sp.		+
B-10	636-11	Pseudomonas sp.		+
B-11	108-6	Pseudomonas sp.		+
B-12	247-A3a	Not identified	Colorless yeast	+
B-13	189-3	Ditto	Ditto	+
B-14	169-10	"	"	+
B-15	189-5	"	"	+
B-16	R-3	Bacillus sp.	Opaque form	+
B-17	R-11	Bacillus sp.	Mucoid form	+
B-18	R-12	Pseudomonas aeruginosa		+
B-19	R-1	Not identified	"Green fungus"	+
B-20	R-4	Hormodendron sp.	"Brown fungus"	++[d]
B-21	R-5	Not identified	"Gray fungus"	++
B-22	R-6	Ditto	"Yellow-green fungus"	-
B-23	R-7	Aspergillus sp. (?)	"Black fungus"	+
B-24	NRL-HC-20b	Penicillium sp.		+
B-25	NRL-FC	Hormodendron sp.		-
B-26	NRL-FH	Hormodendron sp.		+++[d]
B-27	NRL-F-2b-30	Fusarium sp.		++[d]
B-28	NRL-HC-22	Aspergillus sp.		-
B-29	QM-7828	Cladosporium resinae f. avellaneum		+++[d]
B-30	QM-7998	C. resinae f. avellaneum		+++
B-31	QM 012	C. resinae		+++
B-32	QM 013	C. resinae f. avellaneum		+++
B-33	QM-8177	C. resinae		+++
B-34	QM-7829	Paecilomyces variotii		+[d]
B-35	QM-8009	P. variotii (var.)		+
B-36	QM-8015	A. terreus		+
B-37	QM-8011	Syncephalastrum racemosum		-
B-38	QM-8014	Chaetomium indicum		-
B-39	28a	Not identified		++[d]
B-40	28b	Pseudomonas sp.		++[d]
B-41	30	Pseudomonas sp.		-
B-42	38	Pseudomonas sp.		+++[d]
B-43	115a	Not identified[e]		+++[d]
B-44	110b	Pseudomonas sp.		+++
B-45	175Bd	Not identified		+
B-46	186b	Serratia sp.		+
B-47	186c	Pseudomonas sp.		+
B-48	188Ab	Pseudomonas sp.		++
B-49	190a	Not identified		+++[d]
B-50	190Aa	Ditto		++
B-51	199b	Pseudomonas sp.		++

TABLE 2. (Continued)

Battelle Code No.[a]	Other Code No.[b]	Identification	Other Information	Growth in Fuel Culture System at 30 C 14 days[c]
B-52	202a	Not identified		++
B-53	327Hb	Aerobacter aerogenes		+
B-54	RB1	Pseudomonas sp.		+++[d]
B-55	--	Hormodendron sp.		+++[d]
B-56	17 N. E. Tank	Not identified	Gram +, rod	+
B-57	18 H. T.	Ditto	Gram +, diplococcus	+
B-58	III N. E. Tank	"	Small gram - rod	+
B-59	55OT-22, 308 Sinc.	Pseudomonas sp.		+
B-60	55OT-22, 251 No. 1 Sinc.	Not identified	Short rod	+
B-61	20 U. N. E. Tank	Ditto	Small gram - rod	+
B-62	"Polyester culture"	"	Bacterium	+++[d]
B-63	"Aluminum culture"	"	Ditto	+++[d]
B-64	"Zinc-Iron culture"	"	"	+++
B-65	"Methane bacteria culture"	"	"	+++
B-66	None	Pseudomonas aeruginosa	"	+++[d]
B-67	"	Hormodendron cladosporoides		+++[d]
B-68	"	Clostridium sporogenes		-
B-69	"	Spirerotilus natans		-
B-70	"	Desulfovibrio desulfuricans		-
B-71	UD-1	Not identified	Gram - coccus	-
B-72	UD-2	Ditto	Gram +, coccus	-
B-73	UD-4	"	Gram +, rod	-
B-74	UD-5	"	Gram +, rod	-
B-75	UD-6	"	Gram +, rod	-
B-76	UD-7	"	Gram +, rod	-
B-77	UD-8	"	Gram +, rod	-
B-78	UD-9	"	Gram -, coccus	-
B-79	UD-11	"	Gram -, rod	-
B-80	UD-12	"	Gram -, rod	-
B-81	UD-16	Streptomyces sp.		-
B-82	UD-17	Actinomyces sp.		-
B-83	UD-18	Not identified	Gram -, rod	-
B-84	UD-19	Arthrobacter sp.	Gram -, rod	-
B-85	UD-20	Not identified	Gram +, rod	-
B-86	UD-21	Ditto	Gram +, rod	+
B-87	UD-22	"	Gram +, rod	-
B-88	UD-23	"	Gram +, rod	-
B-89	UD-24	"	Gram +, rod	-
B-90	UD-25	"	Gram +, rod	-
B-91	UD-26	"	Gram +, rod	-
B-92	UD-27	"	Gram +, rod	-
B-93	UD-28		Gram +, coccus	-
B-94	UD-29		Gram +, coccus	-
B-95	UD-30		Gram +, rod	-
B-96	UD-31		Gram +, rod	-
B-97	UD-32		Gram +, rod	-
B-98	UD-33		Gram +, rod	-
B-99	UD-34		Gram -, coccus	+

TABLE 2. (Continued)

Battelle Code No.[a]	Other Code No.[b]	Identification	Other Information	Growth in Fuel Culture System at 30 C 14 days[c]
B-100	B-1	Aerobacter aerogenes		+++
B-101	B-2	Escherichia coli		-
B-102	B-3	Pseudomonas aeruginosa		-
B-103	B-5	Staphylococcus aureus		-
B-104	B-8	Bacillus cereus		-
B-105	B-9	Bacillus subtilis		-
B-106	B-11	Proteus vulgaris		-
B-107	B-12	Pseudomonas fluorescens		-
B-108	B-13	Sarcina lutea		-
B-109	B-14	Serratia marcescens		-
B-110	B-15	Staphylococcus albus		-
B-111	B-17	Micrococcus radiodurans		-
B-112	B-18	Not identified	Aluminum corrosion No. 308	-
B-113	B-19	Ditto	Aluminum corrosion No. 304	-
B-114	B-20	Flavobacterium arburescens		-
B-115	B-21	Sphaerotilus natans		-
B-116	B-22	Clostridium sporogenes		-
B-117	Y-1	Rhodotorula rubra		+
B-118	F-1	Spicaria violacea		-
B-119	F-2	Fusarium roseum		-
B-120	F-3	Aspergillus niger		-
B-121	F-4	Cladosporium resinae		+++
B-122	F-5	Aspergillus tamarii		-
B-123	F-6	Penicillin orchrochloran		-
B-124	F-7	Alternaria tenuis		-
B-125	F-8	Fusarium moniliforme		-
B-126	F-9	Chaetomium globosum		-
B-127	UD-15	Not identified		-

(a) Coded as received.

(b) The sources for the cultures were:
B-1 through B- (The Boeing Company, Wichita)
B-16 through B-23 (Mellon Institute)
B-24 through B-28 (Naval Research Laboratory)
B-29 through B-38 (Quartermaster Research and Engineering Center)
B-39 through B-55 (U. S. Army Biochemical Laboratory)
B-56 through B-61 (Quartermaster Research and Engineering Center)
B-62 through B-65 (Thermoline Company)
B-66 through B-67 (The Boeing Company, Seattle)
B-68 through B-69 (The University of Dayton)
B-70 (65?0th Aerospace Medical Research Laboratory)
B-71 through B-127 (The University of Dayton).

(c) The symbols represent:
- = no apparent growth
+ = slight growth
++ = moderate growth
+++ = good growth

(d) Evaluated in pure-culture studies of elastomer deterioration.

(e) Tentatively identified as Pseudomonas sp.

kind of culture. The slants were prepared by heating B-H agar medium so that the agar was dissolved, pouring 40 milliliters of the medium into the bottles, autoclaving the loosely capped bottles at 121 C for 20 minutes, and then placing the bottles at an appropriate angle before the agar had set. When the agar had set, 40 milliliters of JP-4 jet fuel (sterilized by Seitz filtration) containing 0.15 per cent anti-icing additive 98/2* (Union Carbide Ucar Fuel-Additive) was aseptically introduced into the bottle by means of a Filamatic automatic pipetter. Duplicate transfers of the cultures were made, the bottles incubated at 30°C, and observations of growth made at various times up to 28 days.

Subsequent transfers of the cultures were made with the above culturing system, except that sterile elastomer strips (described in the following section) were included in the culture setup. This was done to assure the continuing polymer-degrading capability of the cultures. All subsequent transfers of stock cultures were made in this manner. The culture system is described in Figure 1. Typical fungus and bacterial growths are shown in Figures 2 and 3.

In addition to being cultured in the above bottle culture system, the isolates were carried on test-tube slants of tryptone glucose extract (TGE) + 0.1 per cent Difco yeast extract, or other special laboratory media. Media used in these studies are given in the Appendix (page 99).

The B-H mineral salts medium recommended for use in these studies is satisfactory in most respects. However, two disadvantages of the medium are (1) lack of minor elements which are often essential to optimum microbial growth and (2) a precipitate of moderate proportions which makes difficult visual evaluation of the degree of microbial growth in this medium. The precipitate is probably composed principally of insoluble iron salts. For the above reasons, several micrometabolic solutions containing iron were considered as a supplement for the B-H medium and as a substitute form of iron. The one finally chosen was a modified version of the "micrometabolic element solution" of Hoagland and Arnon**, containing a chelated form of iron***. This solution has been used in other microbiological studies, and is relatively easy to formulate in comparison with others considered. The precipitate problem in the B-H was minimized but not completely eliminated with this supplement. Comparative studies were not conducted, but the presence of the minor elements undoubtedly provided a better medium for microbial growth. This supplement is described in the Appendix in the section entitled Laboratory Media. The modified B-H was used as a stock culture medium and as a medium in all subsequent experiments. It has been noted that the B-H medium has a moderate buffering effect.

All isolates have grown well on the TGE nutrient agar or appropriate special media. However, as noted in Table 2, more than half of the isolates grew only slightly or not at all when introduced into a culture system containing jet fuel. It is believed that the reason for this is that although most of these microorganisms were isolated originally from kerosene-type fuels, they lost the ability to utilize fuel as a nutrient source when cultured on standard laboratory media such as TGE.

Sixteen isolates were selected, on the basis of their ability to grow in the above culture system, for further pure culture study of the deterioration of selected sealants and coatings. This is described in a later section.

*Additive contains 98 per cent ethylene glycol monomethyl ether, 2 per cent glycerine.

**Hoagland, D. R., and Arnon, D. I., University of California Agriculture Experiment Station Circular No. 347 (1938).

***Requestrene 330, Geigy Chemical Company.

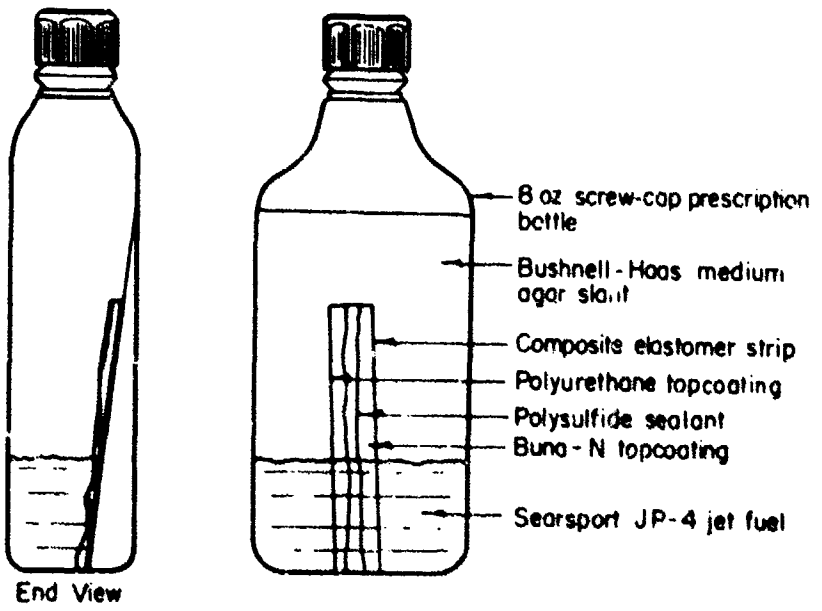

FIGURE 1. DIAGRAM OF STOCK-CULTURE SYSTEM USED TO ADAPT MICROORGANISMS TO GROWTH IN SEARSPORT JP-4 JET FUEL IN THE PRESENCE OF COMPOSITE ELASTOMER STRIPS

N98104

FIGURE 2. STOCK-CULTURE FUNGAL GROWTH (B-29, Cladosporium resinae forma avellaneum)

Stock-culture system diagrammed in Figure 1.

N98103

FIGURE 3. STOCK-CULTURE BACTERIAL GROWTH (B-46, Pseudomonas sp.)

Stock-culture system diagrammed in Figure 1.

Attempts were made to adapt nonjet-fuel isolates, namely Clostridium sporogenes (B-68), Sphaerotilus natans (B-69), and Desulfovibrio desulfuricans (B-70) to "normal" growth in fuel. These attempts were not successful. The observation was made that the addition of Searsport JP-4 (10 per cent by volume) jet fuel to API sulfate-reducing broth apparently did not affect the ability of D. desulfuricans to grow; but, the ability to reduce sulfates (as judged by the development of black precipitate in the medium) was completely inhibited. Since this was outside the scope of the project, no further investigation of this observation was made.

Specimen Sterilization

Elastomer strips used in adapting the growth of microorganisms for polymer-degradation tests were prepared by casting a polysulfide sealant onto 1.0 x 7.5-cm pieces of 0.050-inch aluminum. After the sealant had cured, Coatings A (Buna N) and B (urethane) were individually brush coated along the edges of the sealant-coated aluminum strip. Thus, the strips consisting of a sealant and two different coatings present surfaces for microbial growth. These and other coatings used in these studies are coded in Table 1.

The adaptation strips were placed individually in glass test tubes which were stoppered with Bacti-Caps*. The strips were then sterilized by a 4-hour exposure to ethylene oxide at 57°C and 6 to 7 psi. Sporedex** strips containing viable spores of Bacillus subtilis var. globigii were placed in several tubes along with the adaptation strips so that it could be determined whether the ethylene oxide penetrated satisfactorily into the tubes. This bacterial species is particularly resistant to ethylene oxide sterilization. Following sterilization, the Sporedex strips were placed in sterile thioglycollate broth contained in test tubes and incubated at 30°C. No growth occurred in the broth tubes during 4 days of incubation.

Unsterilized elastomer strips, as well as ethylene oxide-sterilized elastomer strips (described above) were individually placed on B-H plus 0.5 per cent glucose agar or nutrient agar (see Appendix) contained in petri plates. The plates were incubated at 26 ± 5°C (room temperature). Only occasional bacterial colonies (unidentified) occurred in plates containing nonsterilized strips, and none occurred in plates containing sterilized elastomer strips. It should be noted that only four of six of the nonsterilized strips yielded microbial contaminants. In general, only a few microorganisms per strip were observed when growth was present. This was not expected because considerable handling occurred during preparation of the elastomer strips. Some polymers may be self-sterilizing during the curing process.

On the basis of these studies, exposure to ethylene oxide was considered to be a satisfactory method of sterilizing these particular polymers for use in maintaining preculture microbial stocks of microorganisms.

Although it was shown that the ethylene oxide method of sterilization satisfactorily killed the scant microbial populations present on polymer surfaces, there was some question concerning its possible effect on specimens that were to be evaluated for microbial deterioration. Commercial ethylene oxide sterilant is a mixture of gases containing

*American Hospital Supply Corporation.
**American Sterilizer Company.

about 11 per cent ethylene oxide as the active component, with the remainder consisting of various halogenated propellants. These gases are possible solvents for certain polymers. Some work reported in the literature* indicates that a commercial ethylene oxide sterilization would attack certain plastics, notably cellulose acetate butyrate and polystyrene, while nylon was unaffected. Visual observation of the polysulfide sealants and polyurethane and Buna N coatings used in the present study showed no evidence of degradation after ethylene oxide sterilization. Hardness measurements also gave no indication of a change in either the coatings or sealant. However, it was found that the water resistance of the polyurethane and Buna N coatings was improved. It may be assumed that this improvement is the result of further cure brought about by exposure at 57°C during sterilization.

It was decided to use nonsterile panel and rod specimens in subsequent experiments because of possible adverse effects on coatings during heat or ethylene oxide sterilization. Other factors considered in this decision were the low microbial populations found on freshly cured coatings and the desirability of simulating field application of the coatings as closely as possible. Care was taken to minimize handling test specimens, thereby limiting contamination during coating application and other manipulations. Rubber gloves rinsed in 70 per cent ethyl alcohol were used during all handling procedures.

Use of nonsterile test specimens was later justified in that less than 5 per cent of noninoculated controls have had volunteer growth, i.e., contamination, when placed in jet-fuel culture systems.

Isolates for Permanent Collection

An effort was made to establish a "permanent" culture collection of jet-fuel isolates. That is, unidentified microorganisms were given at least tentative identification, and identification of others was verified. Thirty-three cultures were included in this collection. These are being maintained on the jet-fuel substrate for future microbiology studies. Most of the bacteria in the culture collection are pseudomonads, e.g. B-39 through B-54, and B-66. The last mentioned is Pseudomonas aeruginosa, which fits the classic characteristics of that organism as described by Bergey**. While many of the bacteria tested are described as pseudomonads, there were some exceptions based on the findings. For example, B-39 had all Pseudomonas characteristics except that it is oxidase negative. Recent literature*** of Pseudomonas taxonomy was reviewed briefly. It was found that there is considerable disagreement in this field. The scheme of Shewan, Hobbs, and Hodgkiss**** appears to be the best available at this time. By this scheme, four unidentified bacterial cultures (B-62 through B-65) were identified as pseudomonads, with one (B-63) being a mixed culture of Pseudomonas sp and Vibrio sp. These four were previously identified, respectively, as "polymer culture", "aluminum-culture", "zinc-iron culture", and "methane bacteria culture". Because of the disagreement among workers in the field, no attempt at species identification was made.

*Tessler, J., "Reaction of the Sterilant, Ethylene Oxide on Plastics", Appl. Microbiol., 9, 256 (1961).

**Breed, R. S., Murray, E.G.D., and Smith, N. R., Bergey's Manual of Determinative Bacteriology, 7th Edition, London, Bailliere, Tindall, and Cox (1957).

***Gaby, W. L., and Hadley, C., "Practical Laboratory Test for the Identification of Pseudomonas aeruginosa", J. Bacteriol. 74, 356-358 (1957).

****Shewan, J. M., Hobbs, G., and Hodgkiss, W., "A Determinative Scheme for the Identification of Certain Genera of Gram-Negative Bacteria, With Special Reference to the Pseudomonadaceae", J. Appl. Bacteriol. 23 (3), 379-391 (December, 1960).

The identification of all fungus cultures was verified as correct. The culture previously referred to as "Gray Fungus" (B-21) was identified tentatively as Hormodendron sp.

Isolation of Microorganisms From Coatings, Sealants, and Fuel

Petri-plate and bottle studies were conducted on individual elastomer components to determine the possible presence of microbial contaminants. The elastomer components were evaluated as received from the manufacturer, i.e., they were aseptically removed from their containers immediately following the initial opening. Sterile glass rods manipulated by means of sterile forceps were used in obtaining samples for the experiments. This was necessary because of the viscous, sticky nature of some of the materials. In petri-plate studies, the components were simply smeared on the surface of the agar. In bottle (4-oz prescription bottle) studies, the glass rods were merely dropped into the sterile liquid medium. It was estimated that 0.5 to 1.0 g of material was delivered to each plate or bottle in this manner.

The media used in the petri-plate studies were B-H agar medium and the same culture medium, supplemented with 0.5 per cent glucose. Similarly, the bottle studies were conducted with B-H liquid medium and also with B-H liquid medium supplemented with 0.5 per cent glucose. Three samples for each component were included in the petri-plate studies, and duplicate samples were used in the bottle studies. The plates and bottles were incubated at 26 ± 5°C (room temperature) and observed periodically for growth.

No obvious growth occurred in the bottle studies, but evaluation was difficult because of precipitated materials from the B-H medium and insoluble materials from many of the elastomer components. For this reason, 0.1-milliliter aliquots of the liquid media used in the bottle studies were pipetted onto nutrient agar contained in petri plates (three replicates), spread over the surface of the agar by means of sterile glass "hockey stick" spreaders, incubated at 26 ± 5°C, and observed periodically.

The results of these experiments are presented in Table 3. Generally, it may be stated that the elastomer components have very low microbial populations. Indeed, no microorganisms were isolated from six of the components (1, 3, 4, 6, 9, and 12), and with one exception (5) only single colonies were isolated from the other components. In addition, microorganisms were not consistently obtained in all studies for any one component. On the basis of these studies, the materials investigated appear to have very low microbial populations, i.e., somewhat less than 1 microorganism per gram.

The microorganisms isolated (six fungi and one bacterium) were cultured on Difco tryptone glucose extract + 0.1 per cent Difco yeast extract. None appears similar to typical jet-fuel isolates.

Aliquots (200 ml) of Searsport JP-4 jet fuel were added to 50 ml of sterile B-H liquid medium contained in 8-oz wide-mouth screw-capped jars and incubated at 28°C. After 2 days of incubation, an accumulation of dustlike particles appeared at the interface of the two liquids. After 14 days of incubation, it appeared that the dustlike

TABLE 3. ISOLATION OF CONTAMINANT MICROORGANISMS FROM SELECTED ELASTOMERS AND ELASTOMER COMPONENTS USED IN JET-FUEL INTEGRAL WING TANKS

Code No.	Elastomer or Component[a]	Growth, 14 days[b]			
		Bushnell-Haas Medium		Bushnell-Haas Medium and 0.5 Per Cent Glucose	
		Petri Plate[c] (Agar)	Bottle[d] (Liquid)	Petri Plate[c] (Agar)	Bottle[d] (Liquid)
1	Buna N	-	-	-	-
2	Two-part polyurethane, Part A	-	+	-	+
3	Two-part polyurethane, Part B	-	+	-	-
4	Polysulfide Sealant No. 1, accelerator	-[e]	-	-[e]	-
5	Polysulfide Sealant No. 1, base	-	-	-	-
6	Polysulfide Sealant No. 2, accelerator	-	-	-	-
7	Polysulfide Sealant No. 2, base	-	-	-	-
8	Polysulfide Sealant No. 3, accelerator	-	+	-	-
9	Polysulfide Sealant No. 3, base	-	-	-	-
10	Polysulfide Sealant No. 4, accelerator	-	-	+	-
11	Polysulfide Sealant No. 4, base	-	+	-	-
12	Repair cement	-	-	-	-

(a) The Polysulfide Sealant No. 1 was a dichromate-cured material, No. 2 and No. 3 were different manganese dioxide-cured systems, and No. 4 was lead dioxide-cured.

(b) Incubated at room temperature (26 ± 5 C); - = no growth and + = growth. With the exception of No. 8, each "+" denotes a single isolate (colony) found on only 1 of two or three replicate plates. Only two isolates were found in two of three replicate plates in "bottle" studies.

(c) Three replicate samples for each experimental material.

(d) Duplicate samples for each experimental material. No obvious growth occurred in the bottle studies. Therefore, 0.1-ml aliquots of the liquid medium were aseptically pipetted and placed on the surface of nutrient agar plates. The 0.1-ml aliquots were then spread over the surface of the agar with sterile hockey-stick spreaders. The growth noted in these columns represents colonies which grew on nutrient agar after 14 days of incubation, which followed 14 days of incubation in the liquid medium.

(e) Formation of racemose crystals led to false positive readings early in this set of studies. The crystals had the appearance of fungus colonies, and "grew" throughout the agar medium as well as on its surface.

particles were "growing" slowly. Approximately 6 months after this study was initiated, the dust accumulation was very sparse. Attempts to isolate microorganisms from this culture system by standard procedures were not successful, thus indicating that this fuel as received was sterile.

Nutrient Study

The experimental work described here was conducted to determine whether materials extracted from cured coatings and sealants could be utilized as nutrients by selected microorganisms. Positive results of such a test would tie down more closely possible reasons for microbial attachment or apparent attack. It would be expected that materials would be extracted from coatings by water and fuel – perhaps a higher degree of extraction would occur in the case of fuel. The use of fuel, however, would be less conclusive since it would be difficult to ascertain whether the extract or the fuel itself was the effective nutrient. Thus, water extracts have been used in this work.

The candidate coatings and sealants were poured individually into 18 x 150-mm Pyrex test tubes, then drained from the tubes. The coated test tubes were dried in an

inverted position for 14 days at 73°F and 50 per cent RH. Following this, 20 ml of sterile Bushnell-Haas mineral salts medium was pipetted into the tubes, and the tubes were capped with sterile Bacti-Cap plastic tube covers. Four such tubes were prepared for each candidate material. Two of these tubes were then placed in an incubator at 30°C and two in an incubator at 60°C. After a 4-day extraction period, the contents of the two tubes at each temperature were pooled in sterile-water dilution bottles. The pH of the extracts was then determined, notations made as to cloudiness or color, and each evaluated for the presence of indigenous microorganisms. Table 4 identifies the extracts and indicates (1) initial pH, (2) whether extraction obviously occurred, and (3) the presence of indigenous microbial population.

The pH of the B-H extracting medium was not markedly changed by the majority of the polymer extracts. However, Coatings A, D, J (primer), L (cure coat), N, and U tended to lower the pH slightly, with values dropping to a minimum of 6.4. Coating L was the only material that significantly raised the pH value. In this case, it was 8.5. Judging by color or suspended particles, about one-third of the extracting solutions contained a crystalline product. Despite careful handling, approximately one-third of the extracts showed evidence of a significant number of microorganisms. Two-thirds had none.

The undiluted extracts as well as 1:10 and 1:100 dilutions of them were evaluated in pure-culture, test-tube experiments as a source of nutrient for Hormodendron cladosporoides (B-67) and Pseudomonas aeruginosa (B-66). The microorganisms were 14-day and 3-day cultures, respectively, which were cultured in Searsport JP-4 jet-fuel (0.15 per cent Ucar 500 added) overlying B-H Agar in the presence of a composite elastomer strip. The fungal and bacterial cells were individually harvested, mixed for 30 seconds with a Waring Blendor, and washed twice by centrifugation, using liquid B-H mineral salts medium. Inocula were prepared by diluting the fungus with sterile B-H until approximately 20,000 cells/ml were in the fungal suspension and approximately 120,000 cells/ml were in the bacterial suspension. Estimates of the microbial populations in the inocula were made with a Hellige hemocytometer for the fungus and by means of petri-plate counts ("hockey stick" spreader technique) for the bacterium.

The undiluted extracts and the 1:10 and 1:100 dilutions were then inoculated with 0.1 ml of either the fungus or bacterial inocula. The tubes were individually shaken by means of a Vorte Junior Mixer, incubated at 30°C, and observed periodically for growth. Since the fungus grew well, it was possible to use a simple rating system for these evaluations. Bacterial growth was not great. Thus, it was not possible to use a similar rating system for these specimens. Therefore, evaluation of growth was made in the lowest dilution (1:100) of each extract and the B-H control tubes by the plate-count method previously described. The results of these experiments are presented in Tables 5 and 6.

A small amount of fungus and bacterial growth occurred unexpectedly in the B-H control medium. Ample nitrogen in the form of NH_4NO_3 is available in this medium. However, lack of a carbon source should be somewhat limiting to growth. The two centrifuge washings should have removed most potential nutrients from the cells, but apparently all were not removed. Even so, an increase in growth over that observed in the controls occurred in certain of the experimental extracts.

TABLE 4. PRELIMINARY DATA ON COATING EXTRACTS USING BUSHNELL-HAAS MINERAL SALTS LIQUID MEDIUM AS THE EXTRACTION SOLVENT

Coating Code	Coating Type	Extraction Temperature[a], C	Initial pH[b]	Obvious Extraction[c]	Microbial Contamination[d]
A	Buna N	30	6.8	No	-
		60	6.4	Yes	
B	2-part polyurethane	30	6.8	Slight	+
		60	6.8	Slight	
C	Polysulfide	30	6.9	Yes	-
		60	6.9	Yes	
D	Polysulfide	30	6.4	Slight	-
		60	6.4	Yes	
E	1-part polyurethane	30	6.8	No	-
		60	6.8	No	
F	1-part polyurethane	30	6.7	No	-
		60	6.7	Slight	
G	1-part polyurethane	30	6.8	No	+
		60	6.7	No	
H	Nylon	30	6.8	No	-
		60	6.6	No	
I	Furan, gray cover coat	30	6.8	No	-
		60	6.6	Slight	
--	I, black undercoat	30	6.8	No	+
		60	6.6	No	
--	I, primer	30	6.6	No	+
		60	6.8	No	
J	Furan, clear	30	6.9	No	+
		60	6.8	Slight	
--	J, primer	30	6.6	No	-
		60	6.6	No	
K	1-part polyurethane	30	6.8	No	+
		60	6.8	No	
L	Inorganic zinc	30	8.5	No	-
		60	8.5	No	
--	L, cure coat	30	6.4	No	+
		60	6.4	Slight	
M	Epoxy	30	6.8	No	-
		60	6.9	No	
N	Epoxy	30	6.6	No	+
		60	6.4	No	
O	Epoxy	30	6.8	No	-
		60	6.8	No	
--	N, O, primer	30	6.8	No	-
		60	6.8	No	
P	Epoxy	30	6.7	No	-
		60	6.8	No	
Q	Epoxy/polysulfide	30	6.8	Slight	-
		60	6.6	Slight	
R	1-part polyurethane	30	6.8	No	-
		60	6.7	No	
S	Fluoropolymer	30	6.8	No	-
		60	6.7	No	
T	Fluorinated silicone	30	6.7	No	+
		60	6.7	No	
--	T, primer	30	6.7	Yes	-
		60	6.7	Yes	

TABLE 4. (Continued)

Coating Code	Coating Type	Extraction Temperature[a], C	Initial pH[b]	Obvious Extraction[c]	Microbial Contamination[d]
U	Epoxy	30	6.8	No	+
		60	6.4	Yes	
--	U, primer	30	6.9	No	-
		60	6.8	No	
V	Epoxy	30	7.1	Slight	-
		60	7.1	Slight	
W	Epoxy	30	6.6	No	-
		60	6.8	No	
--	W, primer	30	6.8	No	-
		60	6.9	Slight	
--	Control Bushnell-Haas mineral salts medium	--	6.8	--	--

(a) Extract time was 4 days.

(b) Measured by means of Hydrion pH Paper (Microessential Laboratory, Brooklyn, N. Y.). Limited amounts of the extracts did not allow taking pH by other means.

(c) As indicated by the presence of color and/or suspended particulate matter.

(d) Determined by means of petri plate counts, using the "hockey-stick" spreader technique.

TABLE 5. GROWTH OF Hormodendron cladosporoides (B-67) IN COATING EXTRACTS AFTER 6 DAYS OF INCUBATION AT 30°C

Coating Code	Extract Dilution[a]	Rating of Mycelial Growth After 6 Days of Incubation at 30°C[b]	
		4-Day Extract, 30°C	4-Day Extract, 60°C
Control	--	+	+
A	Undiluted	++	++
	1:10	++	+
	1:100	-	+
B	Undiluted	-	-
	1:10	+	+
	1:100	+	+
C	Undiluted	-	-
	1:10	-	+
	1:100	+	+
D	Undiluted	-	-
	1:10	+	+
	1:100	+	+
E	Undiluted	+	+
	1:10	+	+
	1:100	+	+
F	Undiluted	++	++
	1:10	++	+
	1:100	+	+
G	Undiluted	+	+
	1:10	+	+
	1:100	+	+
H	Undiluted	+	+
	1:10	+	+
	1:100	+	+
I	Undiluted	++	++
	1:10	++	+
	1:100	+	+
I, undercoat	Undiluted	++	++
	1:10	++	++
	1:100	+	+
I, primer	Undiluted	+	+
	1:10	+	+
	1:100	+	+
J	Undiluted	++	++
	1:10	+	+
	1:100	+	+
J, primer	Undiluted	+	+
	1:10	+	+
	1:100	+	+
K	Undiluted	+	++
	1:10	+	+
	1:100	+	+

TABLE 5. (Continued)

Coating Code	Extract Dilution[a]	Rating of Mycelial Growth After 6 Days of Incubation at 30°C[b]	
		4-Day Extract, 30°C	4-Day Extract, 60°C
L	Undilution	-	-
	1:10	-	-
	1:100	-	+
L, cure coat	Undiluted	-	-
	1:10	+	+
	1:100	+	+
M	Undiluted	++	±
	1:10	++	+
	1:100	+	+
N	Undiluted	++	+
	1:10	++	++
	1:100	±	+
O	Undiluted	++	++
	1:10	+	+
	1:100	±	±
N, O, primer	Undiluted	+	+
	1:10	+	+
	1:100	-	+
P	Undiluted	-	+
	1:10	-	+
	1:100	+	+
Q	Undiluted	-	-
	1:10	+	+
	1:100	+	+
R	Undiluted	++	++
	1:10	++	+
	1:100	+	+
S	Undiluted	±	±
	1:10	±	±
	1:100	±	+
T	Undiluted	±	±
	1:10	±	±
	1:100	+	+
T, primer	Undiluted	-	-
	1:10	+	-
	1:100	+	±
U	Undiluted	+	-
	1:10	+	+
	1:100	+	+
U, primer	Undiluted	++	++
	1:10	+	+
	1:100	+	±
V	Undiluted	-	-
	1:10	+	+
	1:100	+	+

TABLE 5. (Continued)

Coating Code	Extract Dilution[a]	Rating of Mycelial Growth After 6 Days of Incubation at 30°C[b]	
		4-Day Extract, 30°C	4-Day Extract, 60°C
W	Undiluted	++	+
	1:10	±	+
	1:100	+	+
W, primer	Undiluted	-	±
	1:10	+	+
	1:100	+	+

(a) All extractions and dilutions of extracts were made with sterile Bushnell-Haas mineral salts liquid medium.

(b) The rating system used was

- = no growth
± = questionable or very slight growth
+ = slight growth
++ = moderate growth

Immediately following inoculation the extracts in each tube contained approximately 200 fungus [illegible]. Cell-count estimation was made with Hellige hemacytometer.

TABLE 6. GROWTH OF Pseudomonas aeruginosa (B-66) IN COATING EXTRACTS AFTER 4 DAYS OF INCUBATION AT 30°C

Coating Code	Extract Dilution[a]	Rating of Bacterial Growth After 4 Days of Incubation at 30°C[b]	
		4-Day Extract, 30°C	4-Day Extract, 60°C
Control	--	+	+
A	1:100	++	++
B	1:100	+++	+++
C	1:100	++	++
D	1:100	++	++
E	1:100	++	+++
F	1:100	++	++
G	1:100	++	++
H	1:100	++	++
I	1:100	++	+++
I, undercoat	1:100	+++	+++
I, primer	1:100	+++	++
J	1:100	++	++
J, primer	1:100	++	++
K	1:100	++	+++
L	1:100	+++	+++
L, cure coat	1:100	++	+++
M	1:100	+++	+++
N	1:100	+++	+++
O	1:100	+++	+++
N, O, primer	1:100	++	+++
P	1:100	+++	+++
Q	1:100	++	++
R	1:100	+++	++
S	1:100	+++	++
T	1:100	+++	+++
T, primer	1:100	+++	+++
U	1:100	++	+++
U, primer	1:100	++	++
V	1:100	+++	++
W	1:100	++	+++
W, primer	1:100	+++	+++

(a) All extractions and dilutions of extracts were made with sterile Bushnell-Haas mineral salts liquid medium.

(b) The ratings for growth were based on estimates of the bacterial populations in the extracts. Estimates were made by means of petri plate counts in which Tryptone Glucose Extract Agar and sterile glass, "hockey-stick", spreaders were employed. Duplicate counts for each extract-dilution were made.

+ = approximately 5.0×10^5 cells/ml

++ = between 1.0×10^6 and 1.0×10^8 cells/ml

+++ = more than 1.0×10^8 cells/ml (colonies too numerous to enumerate in count plates).

Immediately after inoculation, the extracts in each tube contained approximately 1.0×10^5 cells/ml (estimation made as described above).

The 30° and 60°C extracts of Coatings A, F, I, I (black undercoat), J, K, M, N, O, R, U (primer), and W supported growth of H. cladosporoides to a greater extent (++) than did the B-H control medium (+). Undoubtedly, these polymer systems could provide suitable nutrients for the growth of this particular fungus in integral jet-fuel tanks.

A number of the extracts inhibited growth of this fungus at one or more, but not all, concentrations. These were extracts of Coatings B, C, D, L (cure coat), P, Q, T (primer), and V. The inhibition noted for Coating C may have been due to the presence of dichromate and that for Coating L, a pH effect (8, 5).

The rating system employed for evaluating fungus growth is not entirely satisfactory because of possible subjective errors. Mycelial weight offers a more definite criterion for use in this type of study. However, in attempts to obtain such measurements, dry recovery weights ranged from 5 to 12 mg per 10 ml of medium. It is questionable whether these represent differences great enough to lie outside experimental error. If further experiments of this nature are carried out, sufficient volumes of extract should be used to obtain more significant differences in mycelial weights.

Under the conditions of this experiment, the growth of P. aeruginosa (Table 4) apparently was enhanced by 1:100 dilutions of all of the extracts. Growth was enhanced to a greater extent (+++) by 30° or 60°C extracts from Coatings B, E, I, I (black undercoat), I (primer), K, L, M, N, O, N+O (primer), N, S, T, T (primer), U, V, W, and W (primer). None of the extracts inhibited growth of this bacteria.

Under the conditions of this experiment it may be stated that: (1) the growth of H. cladosporoides was enhanced by extracts of 12 different coating materials, (2) the growth of P. aeruginosa was enhanced by extracts of all 31 of these materials and especially by 21 of them, and (3) only two coatings (I, black undercoat, and N) markedly enhanced the growth of both microbial species. Thus, it may be concluded that materials extracted from polymeric coatings and sealants used in jet-fuel integral aircraft and ground storage tanks add to the total nutrients available for growth and development of large microbial populations often found in these tanks.

Selection of Microorganisms for Exposure Tests

Buna N, polyurethane, and polysulfide topcoatings (Coatings A, B, and D) were used in determining the most active microbial species for exposure tests conducted throughout the program. These coatings were individually exposed in shake culture to the 16 species of microorganisms that have grown best in the stock-culture system. All coated specimens were sterilized by treatment with ethylene oxide under 6 to 7 psi at 57°C as described in the section Leaded Specimen Sterilization.

Pure-Culture System. The culture system employed is diagrammed in Figure 4. The procedure used for preparing the culture system is described below:

(1) 75 ml of modified Bushnell-Haas liquid medium was introduced into 8-oz screw-capped prescription bottles. These were sterilized at 121°C and 20 psi, after which the bottles were loosely capped and allowed to cool.

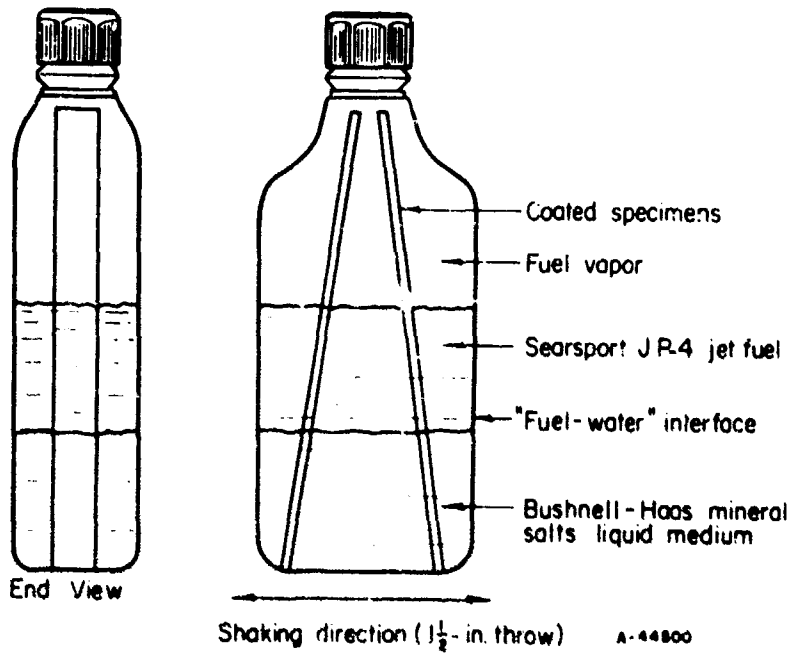

FIGURE 4. CULTURE SYSTEM USED TO EXPOSE INDIVIDUAL ELASTOMERS COATED ON ALUMINUM STRIPS TO PURE-CULTURE GROWTH OF MICRORGANISMS AT 30°C WITH RECIPROCAL SHAKING (90 THROWS PER MINUTE)

(2) 75 ml of Searsport JP-4 jet fuel containing 0.15 per cent Ucar-500 anti-icing additive (sterilized by Seitz filtration) was aseptically added to the bottles by means of a Filamatic automatic pipette.

(3) Pure-culture inocula were prepared by:

(a) Aseptically pouring 20 ml of Bushnell-Haas liquid medium onto microbial growth in stock-culture bottles.

(b) Gently scraping loose the growth with a sterile inoculating needle.

(c) Removing 10 ml with a sterile pipette.

(d) Homogenizing the cell suspension. Homogenization was carried out by blending in a sterile metal cup with a Waring Blendor (low speed for 20 to 30 sec), and by agitation (vibration) with a Vortex Junior Mixer. Very homogeneous inocula were obtained, although optimum mycelial fragmentation was not obtained with fungi.

(4) 1 ml of the inoculum was then aseptically pipetted into the shake culture bottles.

(5) Two sterile specimens coated with the same elastomer were aseptically inserted into each bottle in a manner that would permit them to remain in position during shake incubation.

(6) The bottles were loosely capped (1/4 turn from tightness) and the caps taped to the bottles to keep them in place.

(7) The bottles were then placed in a water-bath shake incubator which was maintained at 30 ± 2°C. The water bath was kept filled to approximately the level of the water-fuel interface in the bottles. The 1-1/2-inch-stroke reciprocal shaking motion was at the rate of 90 cycles per minute.

(8) Observations were made daily for a period of 28 days. Exposed specimens were removed for gross and microscopic examination, pencil hardness, and other evaluations as described in a later section of this report after 3, 7, 14, and 28 days of incubation.

Typical fungus and bacterial growth in the liquid-culture system are shown in Figures 5 and 6.

Generally, it may be said that in this culture system the fungi grew moderately well within 7 days and reached heavy proportions within 14 days. Fungus growth in culture bottles containing the polysulfide sealant specimens was strongly inhibited for about 7 days. Apparently, a fungistatic compound was leached from the polysulfide sealant. After 14 days of incubation, however, attached fungus growth in the interface exposure area of the sealant was easily observable. No inhibition of growth occurred in bacterial culture systems containing this elastomer.

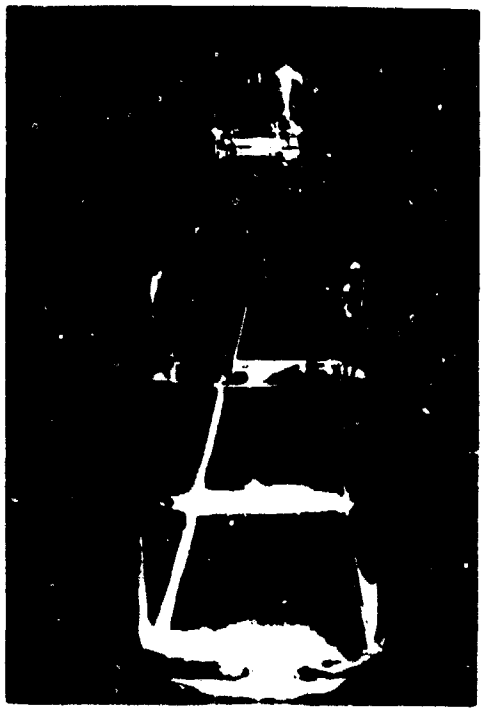

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FIGURE 5. FUNGAL GROWTH (B-29, Cladosporium resinae forma avellaneum) IN SHAKE-CULTURE SYSTEM AFTER 14 DAYS OF INCUBATION

Culture system diagrammed in Figure 4.

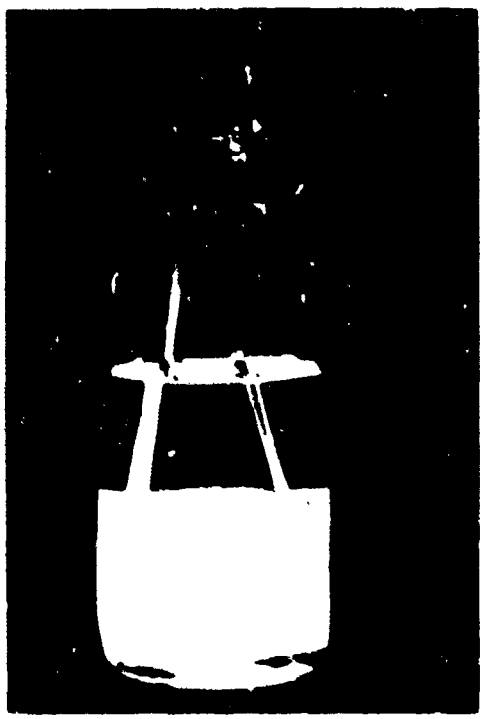

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FIGURE 6. BACTERIAL GROWTH (B-48, Pseudomonas sp.) IN SHAKE-CULTURE SYSTEM AFTER 5 DAYS OF INCUBATION

Culture system diagrammed in Figure 4.

Without exception, all bacteria grew moderately well within 24 hours and reached maximum growth within 3 days in this culture system. Two of the most striking effects noted for several of the bacteria were "blistering" of the polysulfide coating and loss of adhesion of the polyurethane film.

Figure 7 is a diagram showing the arrangement of the coatings in the subsequent figures, as well as the approximate areas of exposure. A photographic record of control and exposed specimens is presented in Figures 8 through 11. The following comments can be made:

Figure 7. The areas of exposure are indicated in an approximate manner because agitation due to shaking resulted in considerable variation in these areas. As a general rule, the water-fuel interface was a "layer" approximately 1 cm in thickness.

Figure 8. This is a fair representation on nonexposed polyurethane and Buna N films. The polysulfide coating varied in texture from that shown in this figure to the one shown in Figure 9, which has a ridged surface. The ridges are not the result of exposure but were formed during application.

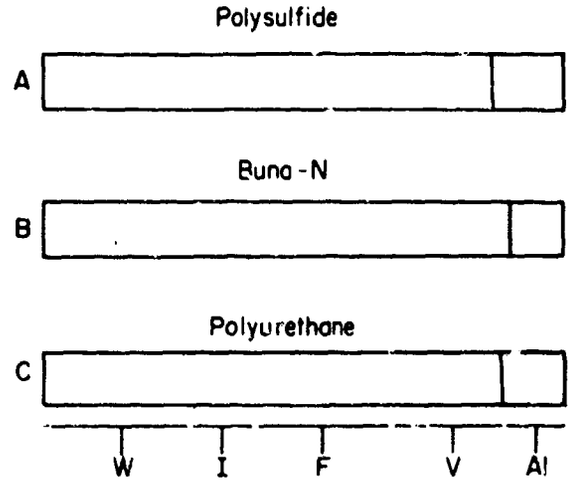
Figure 9. The polysulfide coating and polyurethane film discolored slightly in the water phase and interface areas when exposure was conducted under sterile conditions. This was the only visual change noted for these elastomers in this exposure. The Buna N film responded to exposure to sterile Bushnell-Haas and JP-4 in the following ways:

(1) "Emulsion bubbles" formed in the interface as well as in an adjacent area in the water phase. Some of these interface-area bubbles are clearly shown in the photograph.

(2) The red dye in the Buna N was extracted by the fuel in about 3 days, leaving a clear Buna N film in the fuel-phase area. Small areas of clear film also occurred when emulsion bubbles became attached and persisted in one spot.

(3) The clear Buna N film in the fuel-phase area of exposure became discolored after 7 days of exposure. This discoloration had a tan or tarnished appearance which occurred in the controls and varied in intensity in the various specimens exposed to microorganisms.

Figure 10. This figure illustrates the effect of various bacteria on elastomers. Blistering of the polysulfide also occurred with exposure to growth of B-39, B-42, B-44, and B-49. Increased discoloration in the fuel area of the Buna N (as compared with a similar area in the controls) occurred with the bacterium B-49, and with the fungus B-26. Loss of polyurethane adhesion, which appears in the photograph as opaque areas in the water phase of exposure, occurred with the bacteria B-29, B-43, B-44, and B-49, and the fungus B-55.



Coating Areas of Exposure.

W= "Water "phase (Bushnell - Haas liquid medium containing pure-culture microbial growth).

I= Interface

F= Fuel phase

V= Vapor phase

Al= Bare aluminum strip

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FIGURE 7. DIAGRAM SHOWING ARRANGEMENT OF COATED SPECIMENS IN FIGURES 8 THROUGH 11 AND AREAS OF SPECIMEN EXPOSURE TO CULTURE SYSTEM DIAGRAMMED IN FIGURE 4

Figure 11. The changes shown in this picture are typical of the effect of the fungi evaluated. Attached fungus growth can be observed in the fuel-water interface area. Microscopic fungus growth also occurred in this and the water-phase area. The figure illustrates discoloration in the water and interface areas of the polysulfide specimen. The Buna N specimen does not clearly show the fungal attack that usually occurred, i.e., could be observed growing from them shortly thereafter. Attached fungus growth can be seen in the water-phase area of exposure on the polyurethane specimen. Somewhat less fungus growth occurred in the interface-exposed areas of this elastomer. This may be the result of a "shearing" or "cleaning" action at the rapidly moving water-fuel interface, or it may be an indication that the fungi grow best in quiescent conditions.

Mixed Inoculum. On the basis of the pure-culture studies, five microorganisms were selected for use in mixed-inoculum experiments. These are the pseudomonads B-39, B-43, and B-49, and the fungi B-29 and B-55. Shortly after initiating exposure of coatings to this mixed inoculum, two substitutions were made. B-43 was replaced by B-66 and B-55 by B-67. The reason for the substitutions was that the latter two microorganisms were of the same genus as those replaced, but were more completely identified as to species, and, in addition, had been shown by Kereluk to be deteriorative to fuel tank coatings.

The pencil-hardness method described in a later section of this report (see Methods of Evaluating Polymers) was employed to measure changes in the Buna N and polyurethane coatings. Microorganisms causing the greatest change in hardness, in addition to those causing blistering or loss of adhesion, were selected for the mixed inoculum to be used in all subsequent coating exposures. Hardness evaluations were made as soon as possible after removing samples from the exposure system, while the coatings were still wet. While exposures were carried out primarily to determine the most active microorganisms, it was hoped that preliminary information could be obtained regarding the effect of microorganisms on the coatings themselves. Results of pencil-hardness ratings of coatings exposed to 10 of the more active growing microorganisms are shown in Table 7. Microorganisms which caused the greatest amount of blistering, as well as loss of adhesion or other signs of deterioration, also caused the greatest loss in hardness. On the other hand, it was found that minor imperfections in the coating, as well as differences in film thickness, sometimes resulted in anomalous hardness values. These variations prevent total reliance on pencil hardness as a means of rating microbiological deterioration. A further complication is that several bacterial species formed films or slimes covering the coating which, in some cases, may have affected the pencil-hardness values.

Certain conclusions were reached, however, from a consideration of pencil-hardness values. For example, Buna N coating is known to soften in water, but is relatively unaffected by fuel. Thus, it is considered significant when an isolate such as B-49 causes a marked reduction in hardness of the coating in the fuel and vapor areas. This was the only isolate which showed much effect on the hardness of a Buna N film, although a number of other microorganisms were observed to grow on this coating. The polyurethane coating was altered by four microorganisms: B-39, B-43, B-49, and B-55. These changes were noted as hardness reductions in both water- and fuel-exposure areas.

FIGURE 8. NONEXPOSED ELASTOMER-COATED SPECIMENS

(Refer to Figure 7 for identification.)

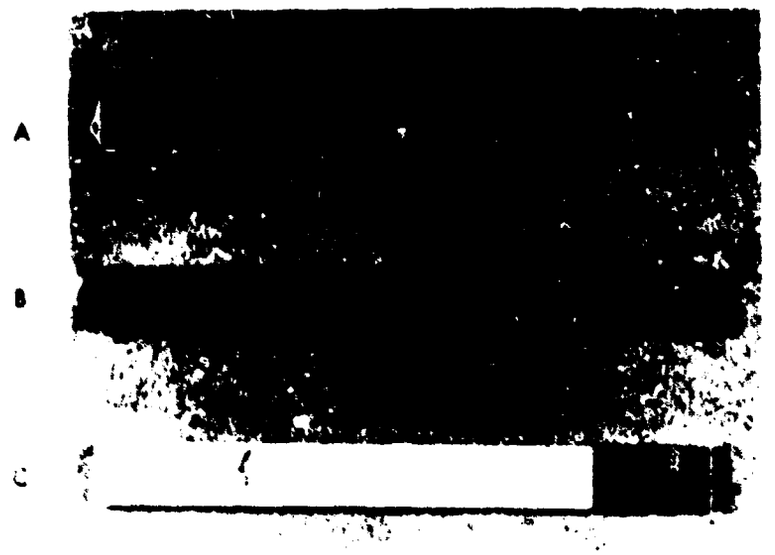

FIGURE 9. ELASTOMER-COATED SPECIMENS EXPOSED TO STERILE "WATER-JET FUEL" SYSTEM. FOUR-WEEK EXPOSURE

(Refer to Figures 4 and 7 for identification and exposure description.)

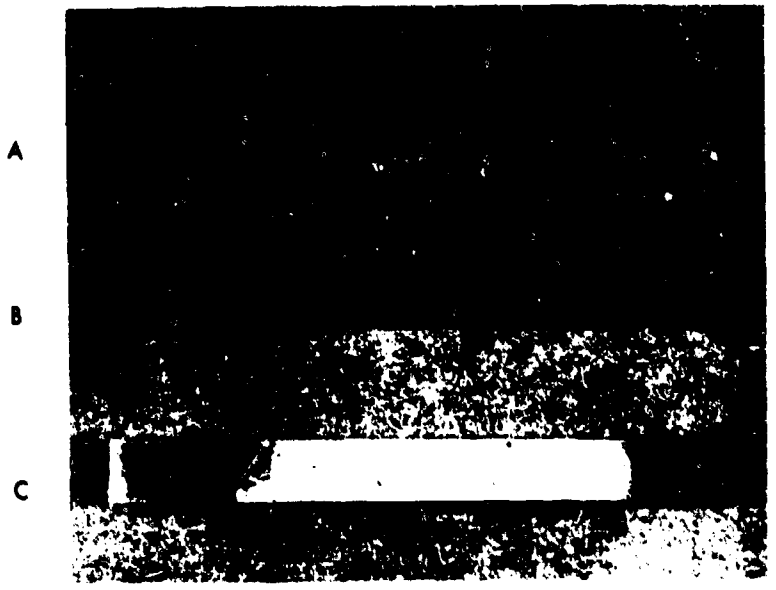

FIGURE 10. ELASTOMER-COATED SPECIMENS EXPOSED TO GROWTH OF Pseudomonas sp. (B-49) IN SHAKE CULTURE. FOUR-WEEK EXPOSURE

(Refer to Figures 4 and 7 for identification and exposure description.)

FIGURE 11. ELASTOMER-COATED SPECIMENS EXPOSED TO GROWTH OF Cladosporium resinae forma avellaneum (B-29) IN SHAKE CULTURE. FOUR-WEEK EXPOSURE

(Refer to Figures 4 and 7 for identification and exposure description.)

TABLE 7. PENCIL HARDNESS OF COATINGS EXPOSED TO PURE-CULTURE GROWTH OF SELECTED MICROORGANISMS[a]

Coating	Isolate Code No.[b]	3 Days				7 Days				14 Days				28 Days			
		Water	Interface	Fuel	Vapor	Water	Interface	Fuel	Vapor	Water	Interface	Fuel	Vapor	Water	Interface	Fuel	Vapor
Buna N	None	4B	6B	HB	HB	6B	6B	HB	HB	5B	5B	HB	HB	3B	3B	HB	HB
(Pencil hardness of	B-39	5B	6B	B	HB	6B	6B	B	B	<6B	<6B	B	B	4B	4B	2B	--
unexposed control	B-42	6B	3B	B	B	6B	6B	B	HB	6B	6B	B	B	6B	5B	B	B
was HB)[c]	B-43	3B	4B	B	B	3B	3B	HB	HB	6B	6B	B	HB	6B	6B	B	B
	B-44	4B	4B	2B	B	3B	3B	B	HB	<6B	<6B	3B	B	6B	6B	B	F
	B-49	<6B	<6B	B	HB	6B	<6B	3B	B	6B	6B	3B	B	<6B	<6B	2B	3B
	B-54	4B	4B	B	B	<6B	6B	HB	HB	6B	6B	2B	B	6B	<6B	B	B
	B-62	4B[d]	4B	HB	HB	6B	6B	B	B	<6B	6B	B	HB	6B	6B	B	B
	B-26					6B[d]	6B	B	HB	6B	6B	2B	B	<6B	6B	B	HB
	B-29					5B[d]	5B	2B	HB	6B	6B	B	B	B	B	B	B
	B-55					4B[d]	5B	B	HB	5B	5B	B	HB	6B	6B	B	HB
Polyurethane	None	3B	3B	B	B	2B	2B	B	B	6B	6B	B	B	3B	3B	B	B
(Pencil hardness of	B-39	2B	2B	B	B	6B	6B	B	3B	5B	5B	3B	3B	5B[d]	5B[d]	5B	<6B
unexposed control	B-42	B	B	B	B	B	3B	HB	HB	3B	3B	B	B	2B	2B	B	B
was HB)[c]	B-43	4B	3B	2B	2B	2B	3B[e]	HB	HB	3B[e]	3B[e]	3B	3B	6B[d]	6B[d]	3B	3B
	B-44	4B	4B	3B	B	B	B	B	HB	3B	4B	B	B	6B[d]	<6B[d]	2B	4B
	B-49	4B	3B	B	B	3B	3B[e]	3B	2B	2B	2B	B	B	<6B	<6B	4B	<6B
	B-54	4B	4B	2B	2B	2B	2B	B	HB	3B	3B	B	B	4B	4B	3B	B
	B-62	4B[d]	4B	2B	2B	3B	3B	B	B	2B	3B	B	B	6B	6B	B	B
	B-26	--	--	--	--	3B[d]	3B	2B	B	3B	3B	B	B	5B	5B	3B	3B
	B-29	--	--	--	--	3B[d]	3B	B	2B	2B	2B	B	B	6B	6B	B	B
	B-55	--	--	--	--	3B[d]	2B	B	B	<6B[e]	3B	B	B	4B[e]	4B	2B	2B

(a) Pencil hardness values were determined immediately after removal of panel from exposure system, while coatings were still wet.

(b) Nos. B-39 through B-54 are Pseudomonas sp.; B-62 is an unidentified bacterium; and Nos. B-26, B-29, and B-55 are fungi of the Cladosporium-Hormodendron group.

(c) Pencil hardness refers to manufacturer's hardness designation from soft to hard as follows: 6B, 5B, 4B, 3B, 2B, B, HB, F, H, 2H, 3H, 4H, 5H, 6H, 7H, 8H, 9H, for the hardest pencil that will not mar the surface of the material tested.

(d) Loss of adhesion from aluminum panel.

(e) Adherent growth on coating.

When exposed specimens were allowed to dry for 3 to 5 days at 23°C and 50 per cent relative humidity, it was found that the hardness of both Buna N and polyurethane coatings returned to their original (unexposed) value. However, two organisms, namely B-39 and B-49 (both pseudomonads), caused permanent softening in fuel- and fuel-vapor- as well as interface- and water-exposed areas. On the basis of these evaluations, the most active microbial species was B-49, which caused permanent softening of 3 pencil units in all exposure areas of polyurethane specimens, and a 1-pencil unit permanent softening in all exposure areas of Buna N specimens. Although pencil-hardness changes were small when dry specimens were evaluated, the ratings obtained may be more indicative of deterioration than wet ratings.

Microbial Resistance of Aluminum Coatings

Preparation of Culture System. The culture system employed for the mixed inoculum evaluations of coatings and sealants is diagrammed in Figure 12. The procedure is similar to the one employed in the pure-culture studies except that aluminum rods rather than panels were used. As in the case of pure cultures, the bottles were placed in a standard laboratory incubator maintained at 30 ± 2°C, following a 3-day shake incubation, and examined monthly. After each inspection, sufficient sterile JP-4 fuel containing the anti-icing additive (Ucar 500) was added to make up fuel lost by evaporation. The volume required ranged from 15 to 25 ml.

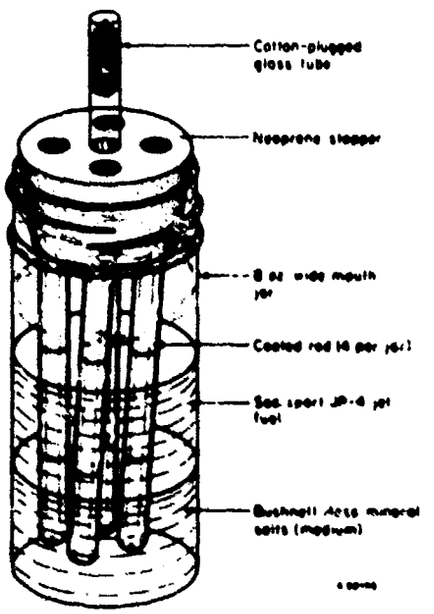

FIGURE 12. CULTURE SYSTEM USED TO EXPOSE COATINGS ON ALUMINUM RODS TO MIXED-INOCULUM GROWTH OF JET-FUEL ISOLATES AT 30°C WITH COMBINED SHAKE-STATIC INCUBATION

Figure 13 is a photograph of the culture system showing how the test specimens were exposed. Figure 14 shows a typical inoculated culture system with attached microbial growth on test specimens after 28 days of incubation.

Long-Term Static Exposure of Individual Coatings. Twenty-six coatings were exposed in the culture system identified above, to the mixed inoculum composed of jet-fuel isolates that had been found to be the most detrimental to selected elastomeric coatings. Coatings tested are listed and identified as to type in Table 1. Each culture system received shake incubation for 3 days followed by shelf incubation at 30°C for the remainder of the exposure period. The exposure media were not changed; hence, these were considered static exposures.

The jet-fuel isolates initially employed in the mixed inoculum were as follows:

Battelle Code No.*	Identification
B-29	Cladosporium resinae f. avellaneum
B-39	Pseudomonas sp.
B-43	Pseudomonas sp.
B-49	Pseudomonas sp.
B-55	Hormodendron sp.

After 3 days of shake incubation at 30°C, the bacteria invariably reached maximum growth in the Bushnell-Haas. The fungi at this time were obviously growing in this medium, but only to a slight-to-moderate degree. After 7 days of total incubation (4 days static), the fungi could usually be observed growing well at the water-fuel interface and on interface-exposed areas of the coated rods. Attempts to obtain reliable cell counts at various stages of incubation were not successful. This was due in part to the varied mixed inoculum employed.

Notations as to the degree of growth in the Bushnell-Haas medium and on the rods at various times during exposure were kept by simple rating systems. These data are partially summarized in Tables 8 and 9.

With the exception of the culture systems in which Coating C was exposed, moderate-to-heavy microbial growth was observed in the B-H medium and at the interface of the jet fuel and B-H within 7 days, and usually before this (3 days). Coating C inhibited growth in the culture systems for 28 to 35 days.

Several coatings appear to be inherently more resistant to the attachment and growth of microorganisms. However, as indicated in Table 8, in time the microorganisms become attached and gradually increase in mass in all cases. The coatings that appear to be somewhat resistant to attachment are Coatings C, I, N, O, and Q. In the case of Coatings N, O, and Q, however, this was true only when they were applied on anodized aluminum. The apparent differences in rate of growth on anodized as compared with Iridited aluminum rods are not consistent for all coatings. No ready explanation for this is available.

*See Table 2 for further information. Substitution made as described on p. 30.

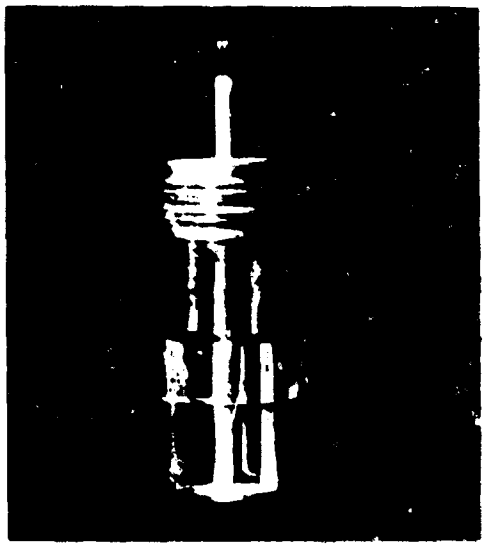

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FIGURE 13. CULTURE SYSTEM PRIOR TO INOCULATION WITH MIXED INOCULUM

Culture system diagrammed in Figure 12.

1704

FIGURE 14. CULTURE SYSTEM AFTER 28 DAYS OF INCUBATION

Culture system diagrammed in Figure 12.

TABLE 8. GROWTH OF JET-FUEL ISOLATES ON COATED 7075-T6 ALUMINUM

Mixed Inoculum Studies in Culture System Consisting of Seaport JP-4 Fuel and Bushnell-Haas Mineral Salts Medium

Coating Code Letter	Coating Type	Rating of Microbial Growth at Indicated Times After Incubation at 30°C[a]			
		14 Days		28 Days	
		On Coating[b]	In Media[c]	On Coating[b]	In Media[c]
A	Buna N	2 (2)	3 (3)	3 (3)	3 (3)
B	Two-part polyurethane	2 (2)	3 (3)	3 (3)	3 (3)
C	Polysulfide	- (-)	1 (1)	1 (1)	2 (1)
D	Polysulfide	3 (2)	3 (3)	3 (3)	3 (3)
E	One-part polyurethane	2 (2)	2 (2)	3 (3)	3 (3)
F	One-part polyurethane	3 (3)	2 (3)	3 (2)	3 (3)
G	One-part polyurethane	3 (3)	3 (3)	3 (3)	3 (3)
H	Nylon	3 (3)	3 (3)	3 (3)	3 (3)
I	Furan (four coat)[d]	1 (3)	1 (3)	3 (3)	2 (2)
J	Furan (two coat)	2 (2)	2 (2)	2 (3)	3 (3)
K	One-part polyurethane	2 (3)	2 (3)	2 (3)	3 (3)
L	Inorganic zinc	2 (3)	3 (3)	2 (3)	3 (3)
M	Epoxy	3 (3)	3 (3)	3 (3)	3 (3)
N	Epoxy	* (2)	3 (3)	1 (2)	3 (3)
O	Epoxy	* (2)	2 (3)	1 (2)	3 (3)
P	Epoxy	3 (2)	3 (3)	3 (2)	3 (3)
Q	Epoxy-polysulfide	1 (2)	3 (3)	2 (2)	3 (3)
R	One-part polyurethane	2 (3)	3 (3)	2 (2)	3 (3)
S	Fluoropolymer	2 (2)	3 (3)	2 (3)	3 (3)
T	Fluorinated Silicone	3 (3)	3 (3)	3 (3)	3 (3)
U	Epoxy	3 (3)	3 (3)	3 (3)	3 (3)
V	Epoxy	3 (3)	3 (3)	3 (3)	3 (3)
W	Epoxy	3 (3)	3 (3)	3 (3)	3 (3)
X	Silicone	3 (3)	3 (3)	3 (3)	3 (3)
Y	Fluoropolymer	3 (3)	3 (3)	3 (3)	3 (3)
Z	Fluoropolymer	3 (3)	3 (3)	3 (3)	3 (3)

Note: No gross deterioration of these coatings occurred after 12 months' exposure in these studies excepting for Coatings C and H (see text). However, in the section on Electrolytic Resistance Measurements of Coatings, it is shown that nearly all of the coatings had significant losses in electrical resistance due to this exposure.

(a) The numbers without parentheses represent ratings of growth observed in culture systems containing elastomer-coated anodized aluminum rods, and those within parentheses were ratings of growth in culture systems containing Iridite-treated aluminum rods. The rating system used was:

- \- = no visible mixed-inoculum microbial growth
- * = questionable or very slight growth
- 1 = slight growth
- 2 = moderate growth
- 3 = heavy growth.

(b) The heaviest growth on the coated rods invariably occurred in the interface area of exposure of the jet fuel-water culture system.

(c) The heaviest growth in the jet fuel-water culture system invariably occurred in the water (or Bushnell-Haas medium) and at the interface of the liquids.

(d) Seven days of cure or drying.

TABLE 9. EARLIEST APPEARANCE OF MODERATE-TO-HEAVY MICROBIAL GROWTH ON EXPOSED COATINGS

Mixed-Inoculum Studies in Culture System Consisting of Searsport JP-4 Fuel and Bushnell-Haas Mineral Salts Medium

Coating Code Letter	Coating Type	Time in Days for Moderate-to-Heavy Microbial Growth to Occur[a]			
		Anodized 7075-T6 Aluminum Rods		Iridite-Treated 7075-T6 Aluminum Rods	
		On Coating[b]	In Media[c]	On Coating[b]	In Media[c]
A	Buna N	3	3	7	3
B	Two-part polyurethane	7	3	7	3
C	Polysulfide	35	28	35	35
D	Polysulfide	7	3	7	3
E	One-part polyurethane	7	3	7	3
F	One-part polyurethane	3	3	7	3
G	One-part polyurethane	3	3	3	3
H	Nylon	3	3	3	3
I	Furan (four coat)[d]	28	3	28	3
J	Furan (two coat)	14	3	14	3
K	One-part polyurethane	7	3	7	3
L	Inorganic zinc	14	7	7	7
M	Epoxy	7	3	7	7
N	Epoxy	56[e]	3	14	3
O	Epoxy	56[e]	3	14	3
P	Epoxy	14	3	7	7
Q	Epoxy-polysulfide	28	3	14	3
R	One-part polyurethane	3	3	14	3
S	Fluoropolymer	3	3	7	3
T	Fluorinated silicone	3	3	3	3
U	Epoxy	3	3	3	3
V	Epoxy	3	3	3	3
W	Epoxy	3	3	3	3
X	Silicone	14	3	3	3
Y	Fluoropolymer	3	3	3	3
Z	Fluoropolymer	3	3	3	3

Note: No gross deterioration of these coatings occurred after 12 months' exposure in these studies except for Coatings C and H (see text). However, in the section on Electrolytic Resistance Measurements of Coatings, it is shown that most of the coatings had significant losses in electrical resistance due to this exposure.

(a) Earliest time at which at least a 2 rating (described in Table 8) was observed. Ratings were made 1, 3, 5 and 7 days and at weekly intervals thereafter.

(b) Occurred invariably at the interface of the jet fuel-water system.

(c) Occurred invariably in the water (or Bushnell-Haas Medium) or at the interface of the jet fuel-water system.

(d) Seven days of cure or drying.

(e) Only slight attached growth was observed on these coatings. The slight growth, first observed on the 28th day, did not progress to moderately heavy proportions.

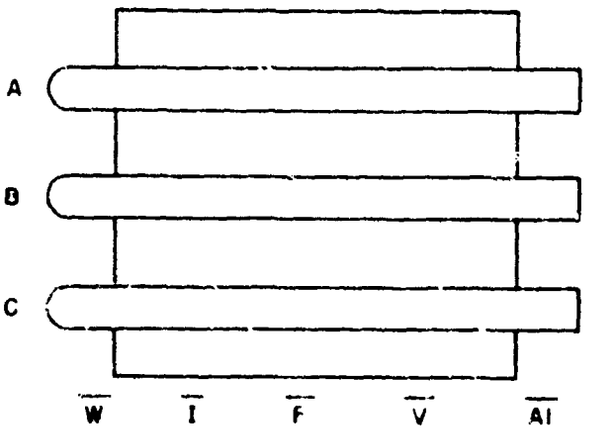
It is recognized that attachment of microbial growth on a coating does not necessarily mean that the coating is being degraded. An electron-microscope study described in a later section showed that, for one type of coating, bacterial cells became embedded in, and fungus mycelia were attached firmly to, the surface of the coating, but on continued exposure there was no apparent further change in the coating.

Following the gross visual observations and removal from culture for picture-taking, the coated specimens were wiped clean, without scrubbing, with gauze wetted with 70 per cent ethyl alcohol and then conditioned for at least 1 week at 50 per cent RH and 72 ± 2°C. A photographic record of selected coatings immediately after removal from the culture systems after 28 days of exposure is presented in Figures 16 through 23. Figure 15 shows the arrangement of coated rods in the exposure tests.

Figures 16 and 17 show Coating A, a Buna N material. Moderate microbial growth is evident in interface areas of coating exposure on both samples of treated aluminum. Figures 18 and 19 show Coating B, a two-part polyurethane. Obvious heavy microbial growth is apparent on both anodized and Iridited specimens, although the location and degree of the attached growth are somewhat different. The reasons for this are not apparent. Figures 20 and 21 show Coating C, a dichromate-cured polysulfide. No visible growth is apparent on either of the rods exposed to the mixed inoculum. This coating immediately imparted a yellowish coloration to the B-H when immersed in the culture medium. Growth on the coating and medium were inhibited. After 28 days of incubation, a slight cloudiness due to bacterial growth was visible in the B-H medium. However, after 35 days of incubation, microbial growth on this coating was rated as at least moderate, thus indicating that the microbial inhibition was not permanent. Figures 22 and 23 show Coating D, a manganese dioxide-cured polysulfide. On specimens in mixed inoculum culture, heavy mixed microbial growth can be observed at the interface area of exposure, and moderate growth in the B-H area. No differences due to the type of aluminum treatment can be seen. As shown in Tables 8 and 9, all other coatings had attached microbial growth after varying incubation times.

After 12 months of exposure to the jet-fuel culture system described, only two coatings showed a significant change. These were the polysulfide Coating C and the nylon Coating H. The observations were made on single specimens only, since the three other replicate specimens had been removed from the system previously for evaluation. The deterioration in both cases consisted of a flaking away of the coating from the substrate, leaving bare areas of aluminum approximately 1 sq mm in area. In both cases, the property changes could have been attributed to the effect of water, since both nylon and dichromate-cured polysulfides were found to be water-sensitive.

Because it was considered desirable not to disturb the standard jet fuel-BH systems in which the coatings were exposed, pH determinations were not made during most of the experiments. Toward the end of the program a set of electrodes capable of measuring pH on one drop of material was acquired. This has allowed determination of the pH with essentially no disturbance of the BH medium containing microorganisms. Drops of the medium were removed from exposure systems by means of sterile pipettes so that the possibility of contaminating the systems was minimized. Results of some of these pH determinations are summarized in Table 10. Keeping in mind that the initial pH of the BH solution was in the range of 6.8-7.0, some generalizations can be made concerning pH change in sterile and inoculated systems containing Coating A (Buna N) and Coating B (a two-part polyurethane) and appropriate controls:



Coating Areas of Exposure:
- W = "Water" phase (Buehnell-Haas)
- I = Interface
- F = Fuel phase
- V = Vapor phase
- Al = Bare aluminum rod

Specimen Coding:
- A = Specimen exposed to mixed inoculum for 28 days
- B = Specimen exposed to sterile media for 28 days
- C = Nonexposed specimen

A-49979

FIGURE 15. DIAGRAM SHOWING ARRANGEMENT OF COATED ROD SPECIMENS IN FIGURES 16 THROUGH 23, AND AREAS OF SPECIMEN EXPOSURE TO CULTURE SYSTEM DIAGRAMMED IN FIGURE 12

C2420

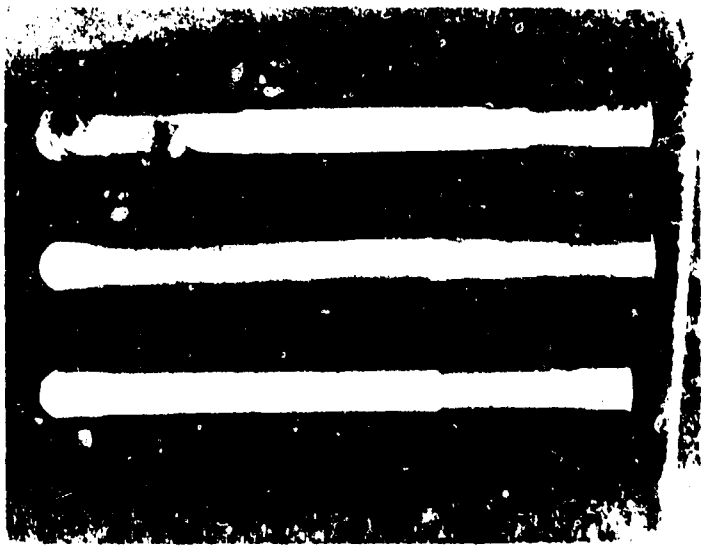
FIGURE 16. BUNA N COATING A ON ANODIZED ALUMINUM RODS, 28 DAYS OF EXPOSURE

Rod identification in Figure 15.

C2421

FIGURE 17. BUNA N COATING A ON IRIDITED ALUMINUM RODS, 28 DAYS OF EXPOSURE

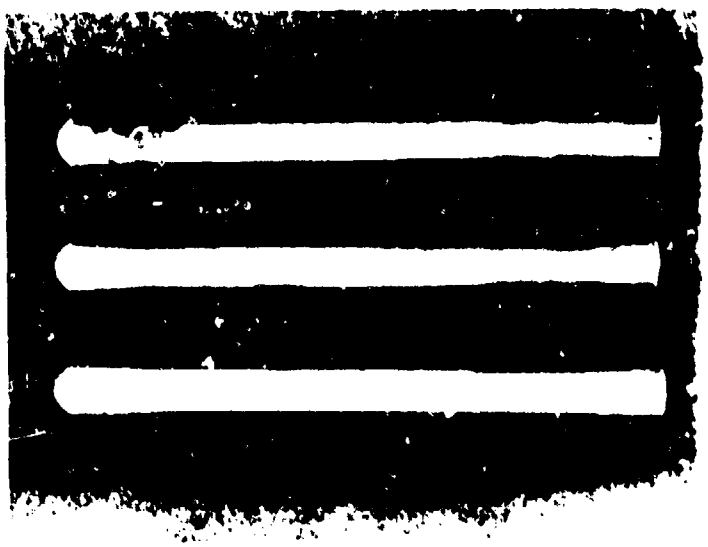
Rod identification in Figure 15.



C 418

FIGURE 18. POLYURETHANE COATING B ON ANODIZED ALUMINUM RODS, 28 DAYS OF EXPOSURE

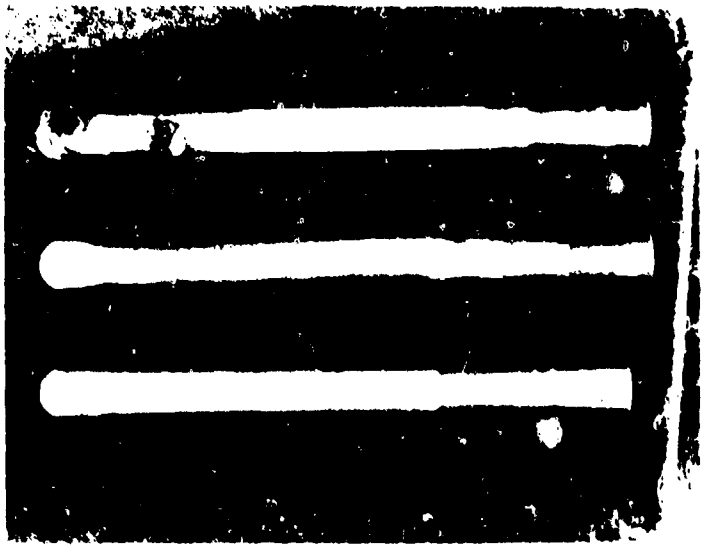
Rod identification in Figure 15.



C241

FIGURE 19. POLYURETHANE COATING B ON IRIDITED ALUMINUM RODS, 28 DAYS OF EXPOSURE

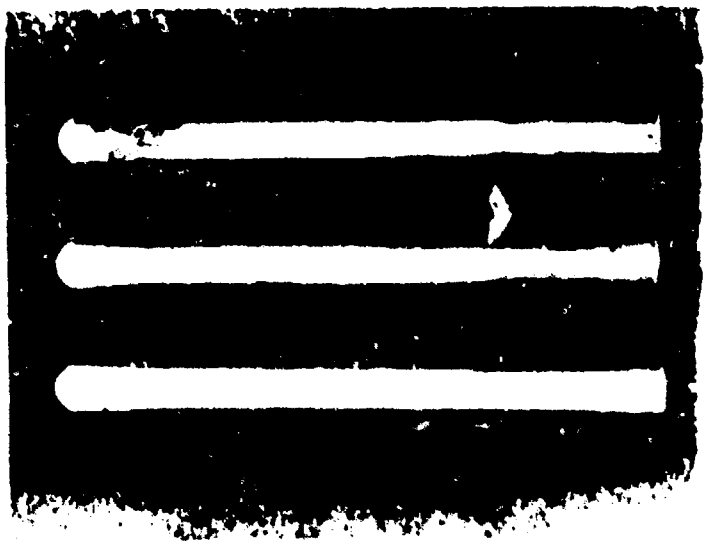
Rod identification in Figure 15.



C 418

FIGURE 18. POLYURETHANE COATING B ON ANODIZED ALUMINUM RODS, 28 DAYS OF EXPOSURE

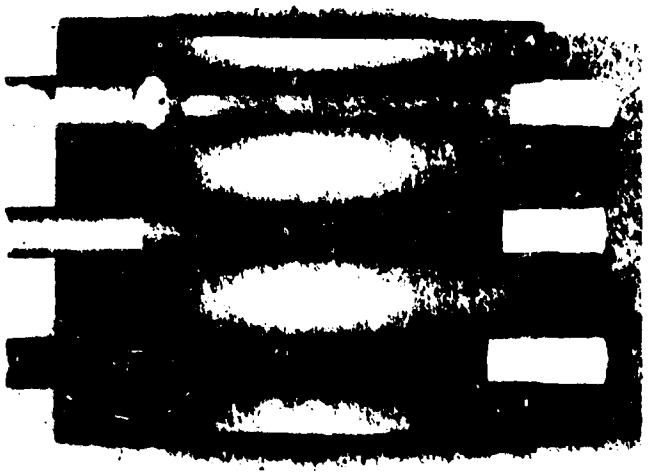
Rod identification in Figure 15.



C841

FIGURE 19. POLYURETHANE COATING B ON IRIDITED ALUMINUM RODS, 28 DAYS OF EXPOSURE

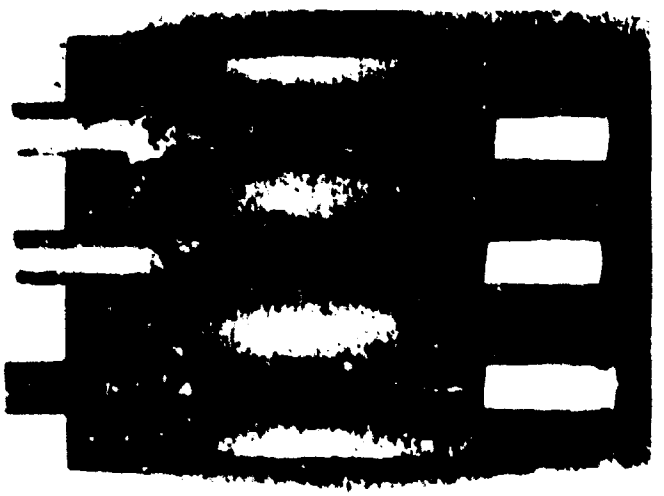
Rod identification in Figure 15.



C2381

FIGURE 22. POLYSULFIDE COATING D ON ANODIZED ALUMINUM RODS, 28 DAYS OF EXPOSURE

Rod identification in Figure 15.



C9382

FIGURE 23. POLYSULFIDE COATING D ON IRIDITED ALUMINUM RODS, 28 DAYS OF EXPOSURE

Rod identification in Figure 15.

(1) Little or no pH change of the B-H medium was found in sterile systems in which no coated rods were exposed.

(2) Slight fluctuations in pH of the B-H medium were observed in sterile exposure systems in which the above-mentioned coatings were exposed for periods of up to 13 months.

(3) Significant changes in pH were observed in inoculated exposure systems containing aluminum rods to which the coatings mentioned above were applied. The pH values of the systems became progressively lower with time, down to a pH as low as 3.20 in 390 days. This was observed for both coatings evaluated.

TABLE 10. pH CHANGE IN MIXED-INOCULUM EXPOSURE SYSTEMS CONTAINING COATED ALUMINUM RODS

Coating Code Letter	Type of Exposure[a]	pH[b] Initial	17 days	180 days	390 days
A	Sterile	6.8-7.0	6.7	7.1	6.7
	Inoculated	6.8-7.0	6.0	5.3	3.2
B	Sterile	6.8-7.0	6.6	7.4	6.7
	Inoculated	6.8-7.0	6.1	5.2	3.3

Note: Culture system consisted of Searsport JP-4 fuel and Bushnell-Haas mineral salts medium.

(a) These readings were taken on various standard exposure systems, which were used also to take ratings for degree of attached microbial growth and to make electrical-resistance measurements. The latter data are reported elsewhere in this report. Both coatings had moderate-profuse attached microbial growth within 28 days, and both had significant losses in electrical resistance after 1 year of exposure.

(b) Average pH of two samples, pH taken with Beckman Model E glass electrode pH meter equipped with 1-drop electrodes.

These rather drastic changes in pH would surely tend to increase the corrosiveness of the B-H medium. It also strongly supports a suspected relationship between growth and corrosivity in aircraft fuel systems. It is noted that the lower part of the pH range of B-H after 6 months of incubation approximates pH readings obtained for the corrosive sump samples collected at Air Force bases and evaluated at Battelle. For example, sumps from Loring Air Force Base and Biggs Air Force Base had pH readings of 4.5 and 4.7, respectively.

Changing-Medium Exposure. Static exposure indicated a need for (1) an accelerated method of exposure, and (2) increased replication of specimens. An attempt to accomplish (1) was made by using the method outlined in the section headed Preparation of Culture System except that the coated specimens were subjected to entirely new exposure systems on a 21-day schedule. In a rather large experiment, in which 8 replicates were included for each coating, 4 such system changes (5 total exposures to new systems) were completed. Then the final exposure systems with the specimens were placed in an incubator at 30°C, observed periodically for attachment of growth, and evaluated by means of electrolytic-resistance measurements.

This changing-medium experiment was terminated at the end of the program, at which time no changes in the electrolytic resistance of any of the coatings had occurred.

This resulted despite the fact that there was moderate-to-heavy attachment of microbial growth on the coatings during a total of more than 4 months' exposure. This was unexpected since it was believed that the effect of the microorganisms as well as leaching by both fuel and water would tend to accelerate coating deterioration.

An interesting observation was that, following the second system change (third exposure), emulsification was evident in some cases at the interface of the fuel and B-H medium. None occurred in sterile control systems containing these coatings. These were Coatings I, L, P, Q, S, and T. The emulsions were not stable and did not recur. Emulsification probably resulted from the combined effects of initial shake-incubation, leaching of materials from the coatings, and the presence of microorganisms.

Combined Coatings. In certain instances one coating type has been applied over another as a means of achieving desirable characteristics not obtainable with either one alone. Although this is not widely practiced in the aircraft industry, dual application of some coatings is known to be in use on a limited scale. Three experiments were conducted to determine whether Buna N (Coating A) when used in combination with polyurethane-type coatings is more or less susceptible to microbial attachment and deterioration in static exposure. The results of these experiments are presented in Table 11. None of the coatings tested is particularly resistant to microbial attachment. The same may be said for all combinations of coatings evaluated.

Biocidal Effectiveness of Furan-Type Coatings. Coatings I and J were evaluated to determine the microbial resistance of furan-type coatings. Experiments were conducted by previously described methods in which 8-oz prescription or wide-mouth bottles containing the jet fuel-Bushnell-Haas culture system and mixed inoculum were employed. Furan-type coatings had been reported in the literature* to resist microbial growth, and in some cases to sterilize fuel in contact with the coating.

The initial experiment was a continuous reciprocal-shake incubation of Coating I on aluminum panel specimens supplied by RTD. Buna N and polyurethane coatings were included in the experiment for comparison. Briefly, it was found that attached microbial growth could be observed within 3 days on all coating materials and that this growth increased to moderately heavy proportions in 28 days, especially in water-fuel interface areas of exposure. The furan-type coating appeared to be slightly more resistant to microbial attachment than Buna N and the two-part polyurethane.

Another observation made at this time was that a thick emulsion formed in the medium at the water-fuel interface in inoculated-culture exposure systems containing the furan coating, as well as in other culture setups containing Buna N and polyurethane-coated specimens. No emulsions were observed when these coatings were shake-incubated in sterile media. Formation of the emulsion was undoubtedly due partly to the continuous shaking and the production of an emulsifying agent by microbial utilization of fuel or components of the coatings. The consistency of the emulsion was mayonnaise-like, similar apparently to emulsions reportedly found in field inspections of integral jet-fuel tanks.

*McGregor, J. M., Drill Bit, pp 28-31 (January, 1963).

TABLE 11. EARLIEST APPEARANCE OF MODERATE-TO-HEAVY MICROBIAL GROWTH ON EXPOSED COATINGS

Mixed-Inoculum Studies in Culture System of Searsport JP-4 Fuel and Bushnell-Haas Mineral Salts Medium

Coating Code Letters	Time in Days for Moderate-to-Heavy Microbial Growth to Occur at 30°C[a]			
	Anodized 7075-T6 Aluminum Rods		Iridite-Treated 7075-T6 Aluminum Rods	
	On Coating[b]	In Media[b]	On Coating[b]	In Media[b]
Experiment I				
A alone	3	3	3	3
E alone	3	3	5	3
E over A	3	3	5	3
F alone	3	3	5	3
F over A	3	3	3	3
G alone	3	3	3	3
G over A	3	3	3	3
H alone	3	3	3	3
H over A	3	3	3	3
K alone	5	3	5	3
K over A	3	3	3	3
Experiment II				
A alone	5	3	5	3
A over B	3	3	14	3
B over A	5	3	3	3
B alone	7	3	3	3
Experiment III				
A alone	5	3	7	3
E alone	7	3	5	3
E over A	3	3	3	3
A over E	3	3	3	3
G alone	5	3	5	3
A over G	3	3	3	3

(a) Earliest time at which at least a 2 rating (described in Table 8) was observed. Since daily ratings of growth were not made, the days noted are not precise indications of when moderate amounts of growth actually occurred. Presentation of the data in this manner allows some comparison of the coatings evaluated.

All of these coatings and coating-combinations had very heavy microbial growth attached at the interface after 28 days of incubation.

(b) Refer to footnotes in Table 8.

This experiment was repeated in a continuous rotary-shake incubation, using Coating I applied on aluminum panels at Battelle. The results with respect to microbial growth and attachment were essentially the same as those reported above, except that the growth on the furan coating occurred more slowly and less profusely. No emulsions were formed in this experiment although the shaking rate in both experiments was 90 rpm. A possible explanation for the lack of emulsion formation is that the rotary motion causes less agitation of the media than does the reciprocal motion.

The slight variability in experimental results at Battelle, together with reports from other laboratories that this coating was highly resistant to microbial attack, led to the theory that cure time is important in the performance of this coating when exposed to the growth of microorganisms. An experiment was designed to test this theory. The procedure followed is described in the section entitled Preparation of Culture System. Both anodized and Iridited aluminum rods, coated with Coating I, were exposed after 2-hour, 2-day, and 7-day cures at room temperature. Results after 28 days of exposure are given in Table 12. Compared with microbial growth in inoculated controls, it was observed after the 3-day period of shake incubation that growth was severely inhibited in fuel-BH systems containing the 2-hour-cured specimens, only slightly inhibited in the 2-day systems, and not at all in the 7-day systems. In subsequent evaluations up to a 28-day period, growth very slowly increased, particularly at the fuel-water interface, in the 2-hour and 2-day cured systems.

TABLE 12. RATING OF MICROBIAL GROWTH IN CULTURE MEDIA AND ON FURAN-COATED ALUMINUM (ALLOY 7075-T6) RODS AFTER 28 DAYS OF SHAKE-STATIC INCUBATION AT 30°C

Coating I, Cure Time	Rating of Microbial Growth[a]			
	Anodized Aluminum		Iridited Aluminum	
	On Coating[b]	In Media[b]	On Coating[b]	In Media[b]
2 hours	-	+	-	-
2 days	++	++	++	++
7 days	+++	+++	++	++

Note: No microbial growth occurred in sterile (noninoculated) culture media (water or fuel) control systems in which duplicates of the above coated rods were exposed.

(a) The rating system used is:
- = no growth visible
+ = slight visible growth
++ = moderate visible growth
+++ = heavy visible growth.

(b) Refer to footnotes in Table 8.

It may be stated that for short periods of time furan-type coatings appear to be somewhat inhibitory to attachment of microbial growth. This is especially true when this coating is exposed to culture growth very soon after application and is probably due to the presence of leachable biocidal substances in the undercured coating.

Microbial Resistance of Steel Coatings

The program was expanded late in 1963 to include an evaluation of coatings used for steel ground storage tanks and carriers. Six such coatings were exposed to the

standard mixed inoculum in the Searsport JP-4 fuel – Bushnell-Haas mineral salts medium. The coating materials were applied by the fill-and-drain technique to 3/8-inch-diameter, solvent-cleaned and grit-blasted, hot-rolled steel (QQ-S-636) rods. The rods are approximately 5 inches long and rounded at the immersion end, as were the aluminum specimens. The coatings were cured for 7 to 10 days at room temperature as specified by the manufacturer.

Attachment and growth of the microorganisms on the coatings occurred rapidly, in most cases within 3 days. As previously discussed, water extracts of these coatings are satisfactory nutrients, particularly for the growth of the bacterium, P. aeruginosa, and to some extent for the fung ladosporoides. Thus, growth of microorganisms on the surface of the coatings is xpected.

Electrolytic resistance values for the six coatings are shown in Table 13. The method for measuring resistance is described on pages 58 to 60. Each coating was tested on 8 steel rods in each exposure; thus, each resistance value in the table is the average of 8 readings. Initial readings, taken after 3 days in sterile and inoculated exposure, indicate that these materials have about the same electrolytic resistance as coatings for aluminum. (See Electrolytic Resistance of Coatings After Microbial Exposure.) Two coatings (N and V) show slightly lower resistance in inoculated exposure as compared with sterile exposure. This is not sufficient to indicate loss of protection for the steel. Another coating (W) presents an interesting example of how electrolytic-resistance measurement can detect deterioration of the coating. Seven of the 8 specimens of this material in microbial exposure showed little change in resistance after 7 months of exposure. However, one specimen began to show a loss in resistance after about 4-1/2 months. Its resistance continued to decrease to a rather low value compared with the others. After about 6 months it was noted that a blister had raised on this specimen and that corrosion products had formed beneath the coating. There is no way of knowing whether the blister and subsequent corrosion of the steel were caused by microorganisms or whether ion transfer through the coating led to corrosion which in turn raised a blister in the coating. Regardless of the mechanism, it represents a coating failure in microbial exposure.

TABLE 13. ELECTROLYTIC RESISTANCE OF STEEL COATINGS EXPOSED TO STERILE AND INOCULATED JP-4/BUSHNELL-HAAS MIXTURES

Coating		Exposure	Electrolytic Resistance, megohms[b]				
Code	Type	System[a]	3 Days	1 Month	2 Months	4-1/2 Months	7-1/2 Months
I	Furan (4 coats)	Sterile	4000	6,000	13,500	1100	4800
		Inoculated	5500	11,300	13,500	1000	3300
L	Inorganic Zinc	Sterile	[illegible]	0.2	0.2	0.02	0.007
		Inoculated	[illegible]	0.8	0.3	0.02	0.04
N	Epoxy	Sterile	19	53	91	128	24
		Inoculated	50	65	34	6	2
U	Epoxy	Sterile	9	25	49	15	130
		Inoculated	11	35	69	14	220
W	Epoxy	Sterile	26	16	20	13	0.5-35[c]
		Inoculated	24	[illegible]	[illegible]	0.1-10[c]	0.05-10[c]

(a) Exposure system consisted of mixture of JP-4 Fuel and Bushnell-Haas mineral salts medium both sterile and inoculated with standard mixed culture (see p 34). In all cases microbial growth on rods and in the media was rated heavy after 3 days exposure to inoculated systems.

(b) The method of measuring electrolytic resistance is discussed in the section headed Coating Evaluation Methods. Results shown are average of 8 specimens.

(c) Range is shown because 1 sample out of 8 had much lower resistance than the others.

A coating based on the cathodic protection afforded by a zinc compound showed continued low resistance throughout the test. Because of this it is not possible to gage the performance of this material by means of the present electrolytic-resistance apparatus. However, if needed, a modified exposure cell could be constructed that would permit more precise readings of lower resistance values. It would probably be advantageous also to measure these coatings as free films.

Coating-Evaluation Methods

One of the problems arising from this study has been the measurement of changes in coatings caused by microbial exposure. Initially, it was thought that visual observation (either by eye or by microscope) might be effective. However, after the mixed microbial exposures were begun and several months passed with no visible evidence of coating change, it was realized that some other method would have to be found. In addition, a quantitative measurement of changes in the coating was desired. Ideally, the measurement method should not disturb the microbial growth or damage the coating. A number of evaluation methods were investigated – some destructive and others nondestructive. The most promising procedure involved determination of the electrolytic resistance of a coating applied to a metal specimen. The various methods studied and a brief description of each are given below.

Pencil Hardness

In initial studies, changes in coating properties caused by exposure to water, fuel, and microorganisms were measured by pencil hardness. In this method, drafting pencils with conventional hardness designations of 6B, 5B, 4B, 3B, 2B, B, HB, F, H, 2H, 3H, 4H, 5H, and 6H (listed in order of increasing hardness) are sharpened in a standard manner, and pushed across the coating. The hardness rating is taken as the designation of the pencil that just penetrates the coating.

The method is simple, fast, and has good reproducibility. However, some coatings in this program were too soft to be checked by this method. This was particularly true of the polysulfides and the furan-type coatings. Also, the method was destructive and did not permit measurement in areas of small surface imperfections. Since the method could not be used with all coatings, its use was discontinued.

Light Microscopy

Microscopic study of a coating appears to offer the most direct way of showing deterioration. However, visual and microscopic examination of topcoatings on aluminum was difficult because the thin polymer coatings tended to follow (not fill in) microscopic imperfections (striations or pits, for example) in the underlying aluminum surface. Thus, it was very difficult to determine whether a coating surface had been deteriorated by microorganisms or is merely following imperfections in the aluminum surface. In addition, reflectance of light from the aluminum surface back through the thin coating as well as from the surface of the coating also made interpretation of microscopic observations very difficult. Microscopic examination is also time-consuming and suffers from the fact that an observation cannot be quantitatively evaluated.

The microscope can be used in two ways: (1) to show defects in the coating, such as blisters and craters, caused by exposure to microorganism, or (2) to show microbial embedment in the surface – a type of incipient failure which in effect may be a pre-deterioration state. The detection of embedment requires magnification of from 1000 to 1500X. In order to assist the observation of the coatings at these high magnifications of the light microscope, differential staining was investigated as a means of resolving the microorganisms and to provide a basis for depth measurement. The water-soluble stain, crystal violet, was used to color the microorganisms, and an oil-soluble red dye was used to stain the coating. This method was effective. However, the dye acceptance of the various topcoat materials differs widely, and further work would be needed to find the most effective dyes for each coating.

The search for surface changes caused by microorganisms can be carried out at lower magnifications, since it is not necessary to view the microorganisms. However, no significant damage could be noted in 100 to 200X magnifications of coatings exposed to microorganisms for 1 year.

Light-Sectioning Microscopy

This is a new development of conventional microscopy. In this method, two microscope objectives are focused on a small area of a surface. By means of light interference, a profile of the surface of the sample is obtained. This method was explored and judged to be useful for describing heavy surface damage. However, since little change occurred in the coatings during standard laboratory exposures, light-sectioning microscopy could not be applied in this study.

Electron Microscopy

Electron microscopy appears to be a promising method for examining coating surfaces. This method has been explored only with the polyurethane coating (Coating B). However, several interesting observations have been made, including study of attached microbial growth on the surface of the coating, and a comparison of the surface morphology of coatings applied by three different coating methods. The principal disadvantage to electron microscopy is that it is time consuming and costly. However, the method shows promise for early detection of coating change.

A replication technique was used in the electron microscopic examinations. Replicas were prepared by softening one side of a cellulose acetate strip with acetone, pressing this against the coating surface, allowing it to dry, and stripping it from the surface. Replicas of adherent bacterial cells and fungus mycelia as well as the polyurethane surface structure were obtained by this technique.

Shadowed replicas were made from the initial cellulose acetate images by vacuum deposition of platinum on specimens held at a 45-degree angle in the vacuum chamber. Carbon was subsequently evaporated in vacuum and deposited on specimens at normal incidence. The cellulose acetate was then removed with acetone, leaving the thin carbon-platinum replica which was viewed and photographed in the electron microscope.*

*Specimens prepared and photographed by C. W. Mellon, Microscopy Division. The electron microscope used was Model JEM-5, Japan Electron Optics Company.

Polyurethane surfaces of specimens exposed for 2 weeks to pure culture growth of Pseudomonas sp. (B-39) and Hormodendron sp. (B-55) were replicated as described above, along with appropriate control specimens. Electron-micrograph reproductions at 8750X magnification are presented in Figures 24 through 27. The following comments can be made:

Figure 24. Nonexposed polyurethane. The granular appearance is believed to be due to the chromate filler in this elastomer. The uniformity of this surface is good, but several distinct areas of imperfection are apparent.

Figure 25. Polyurethane surface exposed to the water part of a water-fuel system. This is similar to the nonexposed control (Figure 24) except that "erosion" appears to have occurred in the upper right part, and unidentifiable depositions of small particles of material are evident throughout the area photographed.

Figure 26. Polyurethane surface exposed to B-39 bacterial growth in the water portion of a water-fuel culture system. Bacterial cells are easily observable in both photographs (see arrows). The cells appear to be somewhat embedded in the surface of the polyurethane. In both photographs, honeycomb areas near or around bacterial cells may be evidence of deterioration.

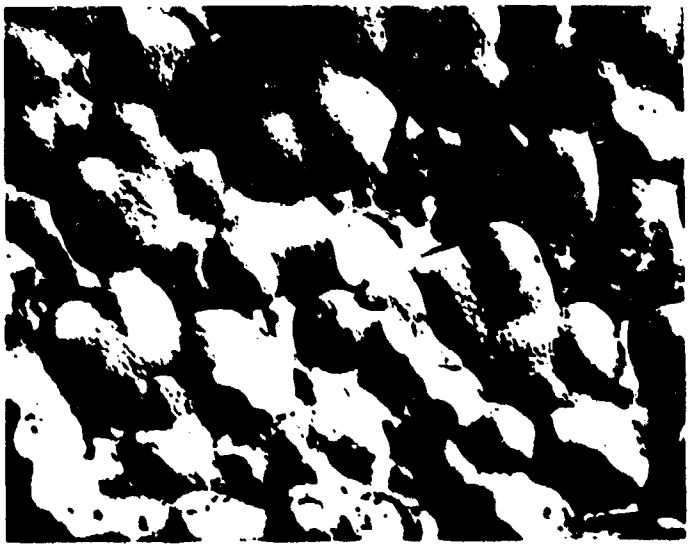
Figure 27. Polyurethane surface exposed to fungal growth (B-55) in the water of a water-fuel culture system. The large fungus mycelia (noted by arrows) occupy major portions of the area in both photographs. "Flaps" at the edge of the mycelia may be the beginning of penetration, i.e., dissolving away, of the polyurethane film by mycelial by-products.

Penetration Tests

The Australian Defense Scientific Service has devised a penetration test to compare the deterioration resistance of various coatings. In this test the coating is used as a membrane separating two media. One medium is sterile and the other is inoculated with a microbial culture system. The time required for microorganisms to penetrate the coating and t appear as growth on the sterile side is taken as a measure of the relative resistance of the coating.

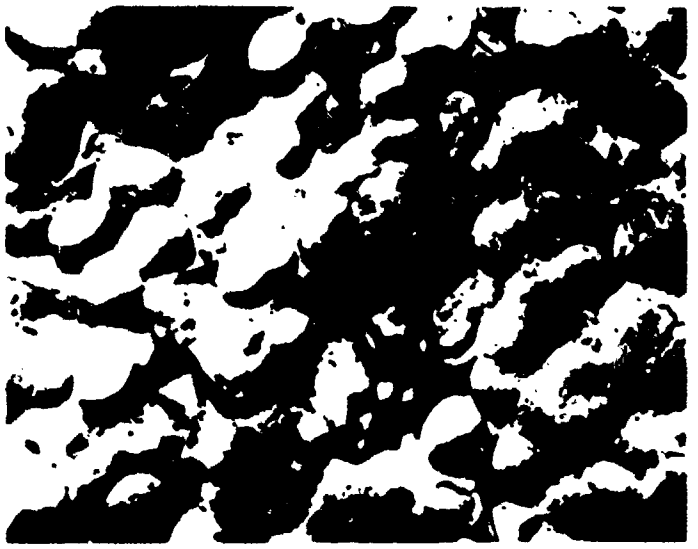
Two variations of the Australian method were studied. The first was proposed by the Coordinating Research Council. In the Australian method, the coating is used as a free film or applied as a coating to nylon cloth. The free or unsupported films are glued over the end of a glass tube that contains the inoculated media. The tube is then immersed in a sterile fuel-water system. The CRC method differs in that the coating is applied over an aluminum screen crimped into one end of an aluminum tube. Drawbacks to this latter method are the variability in thickness of the coating between the wires of the aluminum screen and the difficulty of avoiding small bubbles when applying it.

In the hope of providing a more uniform coating, a second variation was investigated at Battelle. In this method the coating is laid down on a self-releasing surface. It is then removed as a free film and placed over an aluminum disk having a 1/16 or



Approximately 8750X J6955

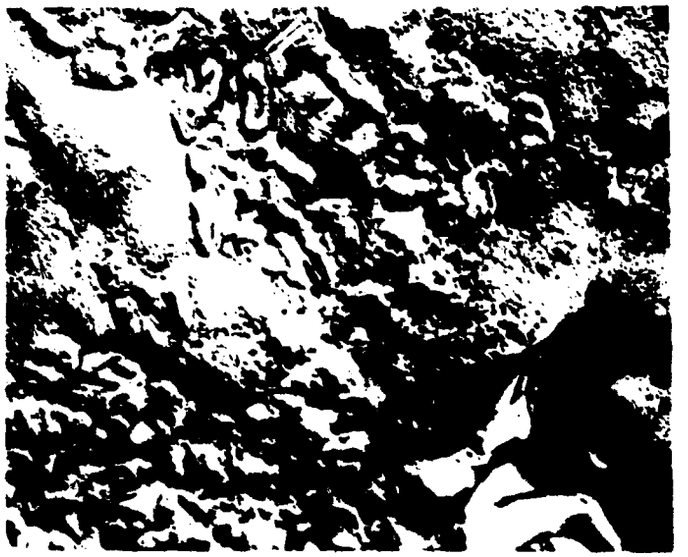
FIGURE 24. REPLICA ELECTRON MICROGRAPH OF NONEXPOSED POLYURETHANE SURFACE



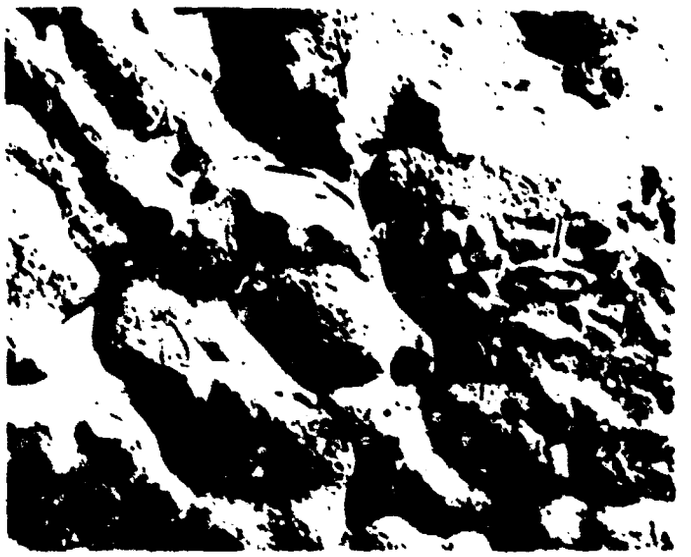
Approximately 8750X J6951

FIGURE 25. REPLICA ELECTRON MICROGRAPH OF POLYURETHANE SURFACE EXPOSED TO STERILE CULTURE SYSTEM DESCRIBED IN FIGURE 4, TWO WEEKS' EXPOSURE

The elastomer surface shown was exposed in the "water" (Bushnell-Haas) portion of the water-fuel system.



Approximately 8750X J6848



Approximately 8750X J6844

FIGURE 56. REPLICA ELECTRON MICROGRAPHS OF POLYURETHANE EXPOSED TO GROWTH OF Pseudomonas sp. (P-38) IN THE CULTURE SYSTEM DESCRIBED IN FIGURE 4, TWO WEEKS' EXPOSURE

The elastomer surface shown was exposed in the "water" (Bushnell-Haas) portion of the fuel-water system. Arrows indicate replicas of one of a group of bacteria cells.

Approximately 8750X J6954

Approximately 8750X J6955

FIGURE 27. REPLICA ELECTRON MICROGRAPHS OF POLYURETHANE SURFACE EXPOSED TO Hormodendron sp. (B-55) IN THE CULTURE SYSTEM DESCRIBED IN FIGURE 4, TWO WEEKS' EXPOSURE

The elastomer surface shown was exposed in the "water" (Bushnell-Haas) portion of the water-fuel system. Arrows indicate fungus mycelia.

1/64-inch hole in the center. The disk is sealed in the end of a glass tube and used in the same manner as in the other penetration tests. Figure 28 is a diagram showing the apparatus for the penetration tests.

Two coatings were investigated – one based on Buna N (Coating A) and the other on a polyurethane (Coating B). A mixed microbial culture consisting of Pseudomonas aeruginosa and Homodendron cladosporoides was used as specified in the CRC procedure. Penetration failures of duplicate coating films were observed in as short a time as 11 days and as long as 82 days. Some tests continued for 150 days without showing penetration. Similar results were experienced with the single-orifice method. It was concluded that both penetration tests are too variable in their present form to be used for the evaluation of coatings.

Moisture-Vapor Transmission

Measurement of the moisture-vapor transmission rate (MVTR) of coating films was explored as a means of detecting changes due to microbial action. The rate of passage of a gas or vapor through a coating is dependent on porosity as well as film thickness. It was felt that any microorganism-induced damage to a film, such as pitting, pin-holing, or even softening and blistering, might show up as changes in the rate of passage of moisture vapor through the film. Moisture vapor was selected rather than some other gas because penetration of the coating by water in service is most likely to result in eventual corrosion of the metal underneath.

Since it is possible to measure the moisture-vapor transmission rate of a film both before and after exposure to microorganisms, a percentage change due to exposure can be obtained. Thus, it is not necessary to be as concerned with the internal bubbles or other defects as in the CRC penetration test.

The moisture-vapor transmission rate was measured as described in ASTM E96-53T, Procedure B. Films of Buna N and polyurethane coatings were cast in thicknesses of 0.5 to 1 mil. The Buna N films were unsupported, but it was necessary to support the polyurethane films by casting them on cellophane. Both coatings were then placed over the top of standard moisture-vapor transmission cups containing water. The assemblies were maintained in a constant-temperature room at 23°C and 50 per cent relative humidity. The cups were weighed daily to record weight loss of water vapor through the film. Typical MVTR values for the films were as follows:

Coating	MVTR, g/24 hr/sq m/mil
Buna N	28
Cellophane	590
Polyurethane on Cellophane	25

There was no apparent change in the MVTR values of the films exposed to microorganisms for 30 days. It is felt that there must be more evidence of coating deterioration before the test could be fully evaluated. However, it is believed that the method could be used in the quantitative measurement of deterioration. The procedure is somewhat cumbersome in its present form but could be improved with the design of special MVTR cups.

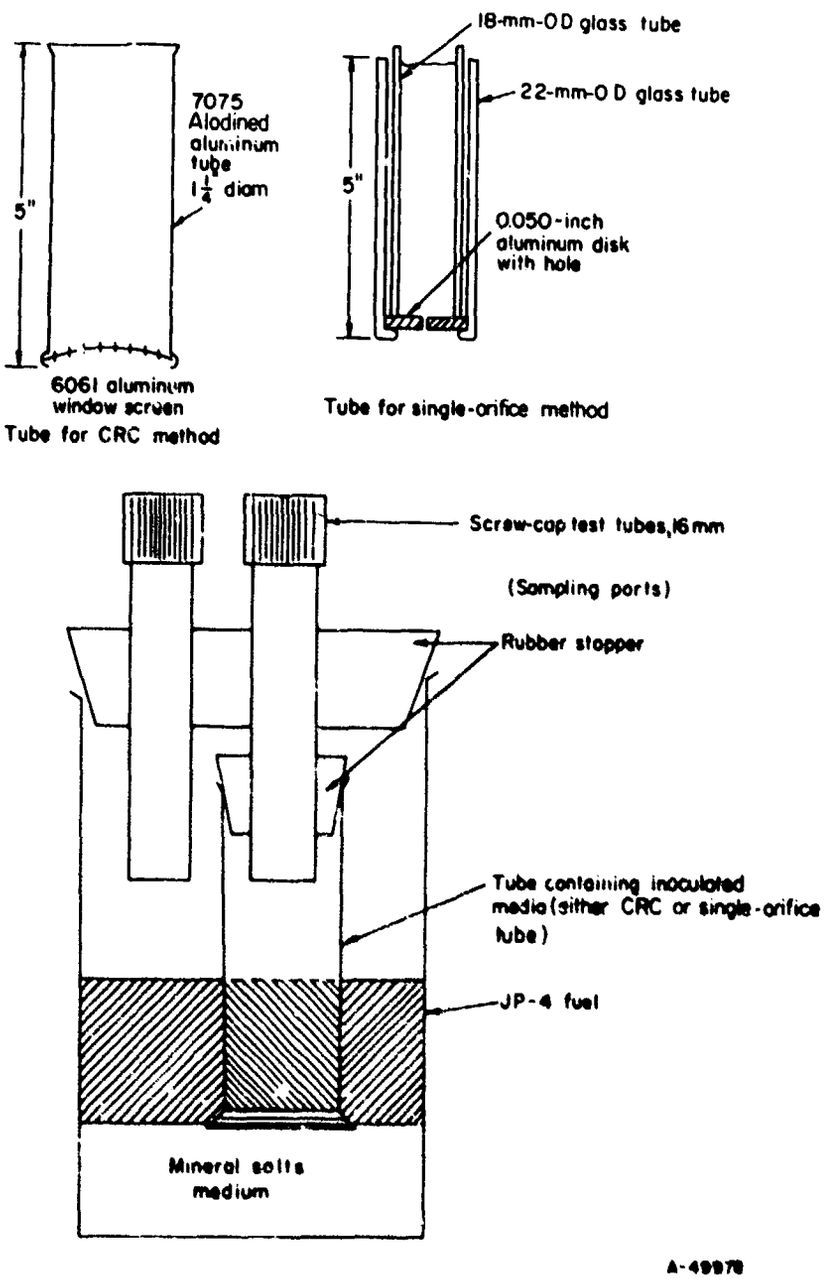


FIGURE 28. APPARATUS FOR COATING-PENETRATION TEST

Fluorescent-Dye Technique

Zyglo (Magnaflux Corporation) is a commercial method for detecting fine cracks in metals. The sample is immersed in a penetrating oil, then exposed to a fluorescent dye which is carried into flaws by the penetrant. Finally, the surface of the specimen is washed clean, removing all dye except that which has been absorbed into flaws. Exposure of the sample to ultraviolet light reveals the flaws. This method was investigated to determine whether the same principle – penetration of surface flaws – could be used to detect microbial damage to coatings on aluminum rods. It was found that little if any surface damage occurred on the coatings as the result of microbial exposure. However, the method did prove effective in revealing imperfections initially present in coatings, suggesting its use to compare the quality of coatings prior to exposure.

Electrographic Printing

Electrographic printing* was explored briefly. In this method, an electric current is passed through a specimen in contact with a sensitized paper. Any pinholes, scratches, or thin spots in the coating permit passage of the current. Aluminum ions from the substrate are deposited on the paper, which contains a dye that is reactive with aluminum. Thus, flaws show up as colored spots immediately over defective areas of the coating. The method appears to lack sensitivity, however. Flaws large enough to be seen by eye were revealed in the electrographic print, but microscopic imperfections did not show up. Nevertheless, electrographic printing made an important contribution by revealing the thinness of the coating at the edge of flat aluminum panels. It was decided that this "edge effect" would make it difficult to compare coatings and, therefore, a change was made early in the program from coated flat panels to coated rods.

Electrolytic Resistance

During this study, the determination of electrolytic resistance of coatings appeared to offer the most promising method for quantitatively measuring the effect of exposure. Experience gained with this method shows it to be sensitive to changes in the coating, and capable of differentiating between the effects of sterile and microbial exposure. The method has the further advantage that the property determined – electrolytic resistance – is directly related to the corrosion protecting capabilities of a coating. This viewpoint is supported by references in the literature, two of which are given below.

Bacon, Smith, and Rugg** have demonstrated that coatings maintaining a resistance greater than 10^8 ohms/cm.^{2} when immersed in sea water gave good protection, whereas if the resistance fell below 10^6 ohms/cm^2, corrosion was rapid. It has been pointed out by Maitland and Mayne*** that corrosion is essentially the conversion of a metal into its oxide, which may or may not dissolve depending on the pH of the environment. The driving force of the reaction is the emf of the metal/oxygen cell which is of the order of 1 to 2 volts. The over-all process can be broken down into two reactions: (1) the anodic reaction, consisting of the passage of ions from the metallic lattice into solution as hydrated cations with the liberation of electrons, which are consumed

*Miller, H. R., and Friedl, S., "Developments in Electrographic Printing", Plating, 47, 520-527 (May, 1960).
**Bacon, R. C., Smith, J. J., and Rugg, F. M., Ind. Eng. Chem., 40, 161 (1948).
***Maitland, C. C., and Mayne, J.E.O., Official Digest, 34 (452), 972 (1962).

elsewhere; and (2) the cathodic reaction involving either reaction of electrons with oxygen and water to form hydroxyl ions, or with hydrogen ions to produce hydrogen molecules. It follows that these processes are accompanied by a flow of current in the metal and the movement of appropriately charged ions in the solution. In order to reduce corrosion, it is necessary to reduce the flow of the corrosion current. This can be done either by retarding the cathodic or the anodic reaction, or by inserting into the electrolytic path of the current a very high resistance, which impedes the movement of ions and thereby reduces the corrosion current to a very small value.

It has been confirmed that organic-coating films are so permeable to oxygen and water that they cannot prevent corrosion by suppressing the cathodic reaction. It has been established that certain pigments may modify the anodic reaction; yet, there are many very effective paint systems that do not contain inhibitive pigments. The view in the latter case is that protection is due to the high electrolytic resistance of a film having a very low permeability to ions.

It was shown by Maitland and Mayne that electrolytic resistance of unpigmented organic-coating films, immersed in solutions of potassium chloride, is controlled by at least three factors:

(1) The temperature – a rise causes a fall in resistance.

(2) The water activity of the solution – as this increases more water diffuses into the film; consequently, more ions from the ionogenic groups present in the polymer become available to carry the current and the resistance falls.

(3) The potassium ion concentration and the pH of the solution – an increase in either favors exchange of the potassium ions with the hydrogen ions derived from the carboxyl groups initially present in the polymer. Since the potassium ions are more easily ionized, the resistance falls.

Kumins* has also demonstrated that certain organic coatings behaved as ion exchange resins. The significance of these references is that they explain the mechanism by which ions are moved across the film, and, in addition, show that it is not necessary to have mechanical imperfections in a coating film for failure to occur.

The role of microorganisms has thus far not been discussed. It is known that microorganisms can attach themselves to the coating surface and can form a mat of growth on that surface. It has been shown in previous work that all coating material submitted for this study will provide nutrients for microorganisms. There is also the possibility of the presence of enzymes or metabolic products from microorganisms, most likely concentrated in areas such as those beneath growth mats. It is possible that the ion-exchange process of a coating film can be increased by microbial action (by the increased formation of ionogenic groups on the polymer molecules). Also, the microbial mat can serve to concentrate gas at the coating surface. Ions in solution may also be concentrated by the microorganisms to accelerate the ion-transport mechanism. A more basic study than the present one would be required to show whether any of these mechanisms occur during microbial exposure.

*Kumins, C. A., Offic. Dig., 34 (451), 843 (1962).

Electrolytic resistance of a coating on metal specimens is determined by momentarily placing an electrode in the jar containing the samples. Thus, readings are taken without disturbing the sample or the microbial growth. The circuit used in such measurements is shown in Figure 29.

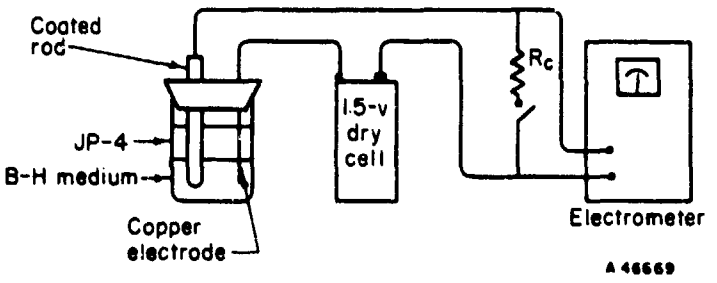


FIGURE 29. CIRCUIT USED FOR MEASUREMENT OF ELECTRICAL RESISTANCE OF COATINGS

Experiments were carried out with various electrodes, including copper, calomel, magnesium, and aluminum. The copper and magnesium electrodes seemed to give the best results from the standpoint of simplicity and adequate cell voltage. Since the copper electrode was used for most of the early measurements, its use has been continued. It was discovered that cell resistance tended to increase steadily over a period of several minutes or even hours as readings were taken, indicating polarization of the metal surface. This reaction, in which aluminum oxide is formed, is undesirable because the Al_2O_3 adds an unknown high resistance to the circuit. Various methods for overcoming polarization were investigated. These included the use of alternating-current measurements and null-type indicators. However, these methods proved to be slower or less sensitive than the procedure now used.

It was found that polarization was due largely to the flow of current permitted by a 1-megohm resistor in the electrometer circuit. This resistor shunted the input terminals whenever the electrometer was switched to a standby condition. When the measurement procedure was changed to avoid this standby condition, polarization was largely avoided. The only other time when a relatively low resistance is shunted across the sample occurs when a standard resistance (R_c) is placed across the cell to obtain the closed circuit voltage. Polarization is held to a minimum at this time by connecting R_c just long enough (about 1 second) for the electrometer to indicate an initial reading. Following this revised procedure, readings could be taken quickly with good reproducibility.

Electrolytic Resistance of Coatings After Microbial Exposure

As indicated in the section on Coating Evaluation Methods, electrolytic resistance measurement of coatings provides an indication of changes taking place during exposure to microbial systems. This is important, particularly when, as in the present study,

no gross visual changes can be observed. The only other means for rating the microb resistance of coatings was that of observing the time for microbial growth to become attached to the coating and to obtain some estimate of the profuseness of the growth. These observations do not reveal changes underneath the surface of the coating and are not necessarily related to the ability of the coating to protect the underlying metal from corrosion. Electrolytic resistance, on the other hand, is related to current flow and hence gives a relative indication of the metal ion flow that might be involved should corrosion occur.

In this program it has been shown that microbial exposure eventually lowers electrolytic resistance of coatings. This is illustrated in Table 14, which shows electrolytic resistance of coatings exposed on aluminum rods for 1 year. Most coatings exhibited no visual evidence of physical damage from the exposure. There were no pinholes, blisters, or other gross imperfections in either the coatings exposed to the microorganisms or those exposed to sterile systems. However, the majority of coatings exhibited decreased resistance in the microbial exposure compared with the sterile exposure.

TABLE 14. ELECTROLYTIC RESISTANCE OF ALUMINUM COATINGS AFTER 1-YEAR EXPOSURE TO STERILE AND INOCULATED JP-4/BUSHNELL-HAAS MIXTURES[a]

Coating		Resistance, megohms			
		Iridited Rods		Anodized Rods	
Code	Type	Sterile	Inoculated[b]	Sterile	Inoculated[b]
A	Buna N	1.04	0.59	3.39	0.2
B	Polyurethane	2.62	0.01	5.62	0.02
C	Polysulfide	0.36	0.01	--	--
D	Polysulfide	0.36	0.01	--	--
E	Polyurethane	3.07	1.03	1.57	0.2
F	Polyurethane	3.10	0.21	4.30	1.2
G	Polyurethane	22.5	1.9	2.7	102
H	Nylon	0.46	0.06	1.01	0.8
I	Furan	--	475	--	--
J	Furan	336	1.02	--	--
K	Polyurethane	65.0	0.88	127	226
L	Zinc	0.12	0.04	0.44	0.04
M	Epoxy	23.3	7.31	5.31	0.59
N	Epoxy	89.0	35.4	113	112
O	Epoxy	95.0	25.4	177	129
P	Epoxy	52.0	28.6	8.4	15.8
Q	Epoxy/polysulfide	0.93	0.45	--	2.2
R	Polyurethane		0.78	--	1.8
S	Fluoropolymer	0.05	0.02	2.8	1.5
T	Fluorinated silicone	765[c]	191[c]	250[c]	48[c]
Y	Fluoropolymer	0.3[d]	0.09[d]	--	--
Z	Fluoropolymer	0.05[d]	0.03[d]	--	--

(a) Coatings differed in thickness and in number of applications. The data are not intended for comparing one coating with another, but to indicate how individual coatings behaved in sterile and microbial exposures.

(b) Exposure system consisted of 1:1 JP-4/Bushnell-Haas mixture, containing the standard microbial inoculum.

(c) Exposed 8-1/2 months.

(d) Exposed 3 weeks.

The specimens in the 1-year exposure were used primarily for visual evaluation of changes due to microbial action. The electrical method was in a development stage during much of this period, and thus no resistance data on these were obtained prior to 6 months of exposure. Also, only one specimen of each coating remained when final readings were taken – the others having been removed earlier for visual inspection.

Measured resistance is influenced not only by the resistance of the coating, but also by the electrolyte, the metal, the oxide layer, and by polarization of the aluminum rod during measurement. In addition, differences in the activity of microorganisms from one container to another, and variations in thickness and quality of coatings make it difficult to assign definite rates of deterioration (as evidenced by resistance values) to samples exposed to microorganisms. However, regardless of these variables, it is significant that in most cases the resistance of coatings exposed to microorganisms is lower than that of similar samples in sterile exposure.

An experiment was carried out to determine whether the lower resistance of coatings exposed to microorganisms is due to changes in the coating, or whether it might be due to the greater conductivity of the system through the accumulation of metabolic products from the microorganisms. This involved three coatings recently removed from a 1-year exposure. The coatings were (1) a two-part polyurethane, (2) a nylon-based material, and (3) a one-part polyurethane applied over Buna N. The specimens were cleaned, sterilized with ethylene oxide, and then placed in a sterile fuel/Bushnell-Haas mixture. The resulting resistance values are shown in Table 15. It can be seen that samples exposed previously to microorganisms maintained a lower resistance than those in sterile exposure; thus, all indications point to the fact that the conductivity of the coatings is increased by microbial exposure.

TABLE 15. ELECTROLYTIC RESISTANCE OF COATINGS REMOVED FROM 1-YEAR EXPOSURE AND PLACED IN CLEAN, STERILE EXPOSURE FOR 10 DAYS

Coating			Resistance, megohms	
Code	Type	Type of Exposure	After 1 Year of Static Exposure to JP-4/B-H Mixture	Ten Days After Exposure of 1-Year Specimens to Sterile JP-4/B-H Mixture
B	Polyurethane	Microbial	0.02	0.10
		Sterile	5.6	6.2
H	Nylon	Microbial	0.06	0.05
		Sterile	9.46	0.18
K over A	Polyurethane over Buna N	Microbial	1.4	26
		Sterile	440	950

Effect of Water and Anti-Icer

While the electrolytic-resistance method has shown promise for determining the effect of microorganisms on individual coatings, it was not possible to use this method to compare the performance of specimens of various coatings prepared earlier in the program. This was due partly to the fact that coatings were not prepared especially for this purpose, and thus were applied in different thicknesses or were otherwise not

standardized. An attempt was made to standardize thickness for at least two coatings that a better comparison could be made. In this study, Buna N (Coating A) and a two-part polyurethane (Coating B) were applied by the fill-and-drain method to both anodized and Iridited aluminum rods. Each coating was given two applications to a thickness of about 5 mils on each rod. After drying for 2 weeks at room temperature, the coated rods were immersed in sterile 1:1 fuel/distilled water mixtures and in 1:1 fuel-distilled water mixtures containing 30 per cent ethylene glycol monomethyl ether in the water phase. This major constituent of the anti-icing fuel additive is often found at concentrations as high as this in the water bottoms of aircraft. Microorganisms were not used in these exposures.

Figure 30 shows the resistance of the two coatings during 15 days of exposure. Further exposure did not change these values appreciably. The results indicate that Coating B (the polyurethane) has higher resistance than the Buna N coating, particularly in the presence of the anti-icing additive. The resistance of Coating A (Buna N) is approximately the same whether on anodized or Iridited aluminum, while Coating B has higher resistance on anodized aluminum. This may indicate that Coating B has greater adhesion to anodized aluminum, while Coating A adheres equally well to both.

There may be several reasons for the increase in electrolytic resistance of samples of Coating A exposed to fuel and distilled water. Extraction of a more conductive component from the Buna N coating by the water would cause greater resistance. Electrolytic reactions at the metal surface (polarization) due to greater current flow through this coating compound could also increase the over-all resistance.

Accelerated Exposure

A method has been devised for accelerating the effect of microorganism exposures. This consists of making a very fine scratch through the coating on the aluminum rod, slightly penetrating the metal surface. These scratched specimens were exposed to the standard JP-4/Bushnell-Haas mixture. Microbial growth tends to concentrate along the scratch area of the specimens. The results of electrical-resistance measurements of these coatings are shown in Table 16. These not only indicate a lower resistance for coatings exposed to microorganisms as compared to sterile controls but also show a reduction of resistance within the first 2 weeks of exposure. Use of the scratch technique therefore appears to offer a means for accelerating the effect of exposure.

TABLE 16. ELECTROLYTIC RESISTANCE OF SCRATCHED COATINGS ON ALUMINUM RODS IN JP-4/BUSHNELL-HAAS MIXTURE AT 30°C

Coating		Aluminum	Exposure	Resistance, megohms[a]		
Code	Type	Treatment	System	1/2 Month	2-1/2 Months	4 Months
A	Buna N	Anodized	Sterile	4.4	2.3	1.05
A	Buna N	Anodized	Inoculated[b]	1.9	0.35	0.21
A	Buna N	Iridited	Sterile	8.8	5.8	1.65
A	Buna N	Iridited	Inoculated[b]	1.5	0.25	0.06
B	Polyurethane	Anodized	Sterile	22.0	11.0	8.75
B	Polyurethane	Anodized	Inoculated[b]	2.8	0.18	1.5
B	Polyurethane	Iridited	Sterile	1.6	0.75	0.14
B	Polyurethane	Iridited	Inoculated[b]	0.9	0.15	0.02

(a) Averaged resistance readings from two exposures of four rods each.

(b) Profuse microbial growth attached to interface areas of coatings within 7 days of exposure. Growth was especially heavy in the scratched area of the coatings within 28 days.

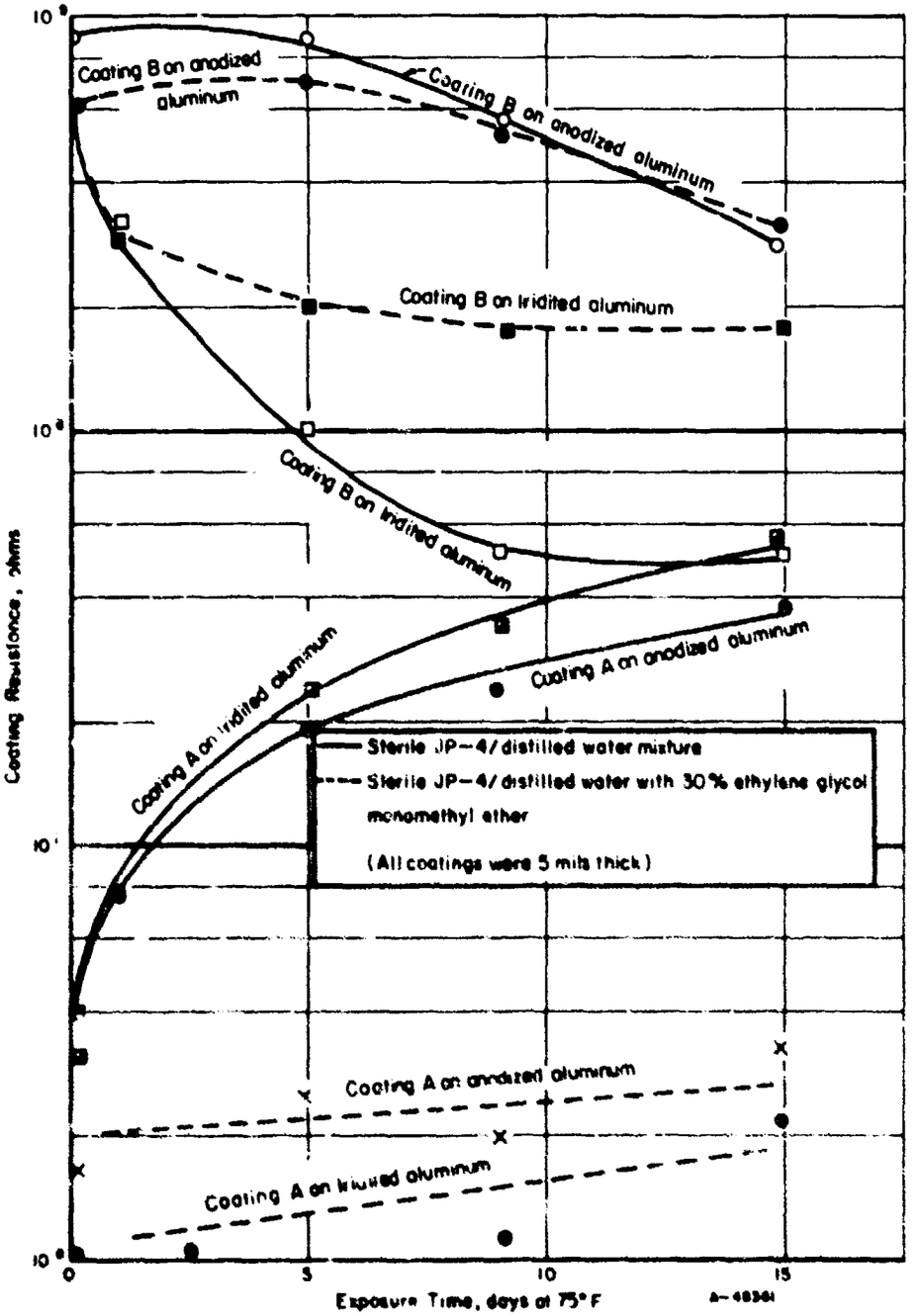


FIGURE 30. EFFECT OF WATER AND ANTI-ICER ON ELECTROLYTIC RESISTANCE OF COATINGS A AND B

Protective Biocides for Aluminum and Steel Coatings

Selection Criteria

A wide variety of biocidal chemicals was obtained for incorporation in Coating B, a two-part polyurethane, to protect it from microbial attachment and deterioration. Selection of this coating was made by RTD. A representative steel coating was also selected by RTD for incorporation of biocides. This is Coating N, an epoxy resin-based material. As shown previously, both coatings are subject to bacterial and fungal attachment, buildup of microbial growth, and loss in electrolytic resistance when exposed to standard systems consisting of P-4 fuel/Bushnell-Haas mineral salts medium and selected microorganisms. Biocides were requested from suppliers with the understanding that any compounds submitted preferably should meet the following criteria:

(1) Low or no water or fuel solubility

(2) No sulfur, halogens, heavy metals, or surface-active compounds

(3) No amine, quinoline, carboxyl, or hydroxyl groups that may affect coating cure

(4) No potentially volatile toxic decomposition products

(5) No strong odors.

Criteria (2) through (5) were based primarily on aircraft-engine and fuel requirements, compatibility with Coating B, and safety considerations based on possible toxicity to humans. These criteria eliminated from consideration virtually all of the more common biocides now on the market. The solubility criterion was established because rapid leaching of the biocide by either fuel or water would render the system ineffective. Since fuel-solubility data were usually not available, this property, as well as solubility in deionized water, was determined at Battelle.

Biocide Permanence

Many of the biocides obtained for incorporation in coatings exhibited a moderate-to-high degree of solubility in either fuel or water, and sometimes in both. As mentioned above, it is believed that the most effective biocides will be those that are relatively insoluble in either fuel or water. In order to determine extractability, biocides were added at a 2 per cent level to three commercial polysulfide sealants conforming to MIL-S-8802C. The compositions were then exposed to fuel and to water for 20 days. Extractability was based on weight loss from each sealant, as compared to controls containing no biocide. Measurements were made on samples weighing about 12.5 grams. Thus, accuracy to 0.1 per cent of the biocide weight was obtained by making weight-loss determinations to 0.002 gram. The results are shown in Table 17. The weight loss in nearly every case accounts for the greater portion of the biocide added. There are variations among the three types of sealants, indicating specific compatibility effects. Some of the variation may indicate chemical reactions between the biocides and sealants. In some cases, a weight loss greater than 2 per cent was noted. This appears to be due to the fact that the biocides altered the cure of the sealant, permitting some additional loss of sealant components.

TABLE 17. EXTRACTION OF BIOCIDE FROM POLYSULFIDE SEALANTS BY WATER AND JP-4 FUEL

Biocide		Per Cent Weight Loss From Sealant After 20 Days of Exposure					
		S-1		S-3		S-2	
Code No.	Chemical Type	JP-4	Water	JP-4	Water	JP-4	Water
Control		2.9	0.8	2.3	0.9	0.9	0.9
SC-1	A nitrotoluene	--	--	4.3	1.5	--	--
SC-3	A modified dioxane	5.3	2.6	4.1	1.7	2.6	2.8
WC-1	A morpholine derivative	5.4	2.5	4.1	1.8	2.7	2.8
WC-2	A piperazine derivative	5.3	2.0	4.1	1.7	3.0	3.6
WC-3	Ditto	5.7	2.7	4.9	2.5	2.7	3.0
WC-4	"	--	--	4.2	1.8	2.1	2.4
GS-2	2-Nitroresorcinol	--	2.6	--	--	--	--
GS-3	8-Hydroxyquinoline salicylate	--	2.5	--	--	--	--
GS-4	Ethylene diacetate	--	2.4	--	--	--	--
GS-5	Alkyl quaternary acetate	--	1.6	--	--	--	--
GS-8	8-Hydroxyquinoline	--	2.2	--	--	--	--
GS-9	Nitrobenzene	--	2.4	--	--	--	--

Note: Sealants were applied as 0.125-inch coatings on 1 x 4-inch panels of 0.050-inch 7075-T6 aluminum. Sealants contained 2 per cent added biocide.

Coatings are used at a thickness of about 0.001 inch compared with 0.125 inch for the sealants. Therefore, measurement of biocide extraction from coatings by weight loss was not attempted. It probably can be assumed that leaching of biocide from coatings would be of the same order of magnitude as from sealants and more rapid. From the experience with sealants it seems reasonably certain that the more soluble biocides need not be evaluated as coating additives, since they would be removed largely by exposure to fuel and water. The decision to concentrate primarily on biocides with limited solubility in water and fuel appears to be correct. If the technique of biocide incorporation in coatings is used, then studies of biocide permanence should be conducted on successful candidate biocides. Either weight loss or bioassay techniques could be used as a measure of biocide loss.

Corrosion of Aluminum by Biocides

Evidence of corrosion or tarnish on aluminum panels protected with coatings containing biocides was noted in the early stages of the program. The tarnish appeared mainly on bare portions of the aluminum rather than beneath the coating. It appeared that the biocides were extracted from the coating during water immersion, and that subsequently the water solution of the biocides attacked the exposed metal. Portions of aluminum protected by the coating, and hence not in direct contact with the water, were tarnished to a lesser degree.

In a study to determine the nature of this corrosion, panels protected on one side with Coating B or Sealant S-1 were exposed to water for 28 days. Eight biocides were evaluated with each of the two coatings, at a concentration of 2 per cent of the dry weight of the coating. Results are shown in Table 18. Two of the biocides caused severe corrosion of aluminum. Three others caused a slight weight loss and would be considered corrosive. Corrosion manifested itself as a darkening and pitting of the aluminum and by the appearance of flakes of aluminum oxide in the water. As with earlier observations, corrosion was more severe on uncoated areas, although some discoloration of

aluminum occurred beneath the coating. If biocides are to be used in coatings, their corrosiveness toward the metals involved should be determined.

TABLE 18. CORROSION OF ALUMINUM BY BIOCIDES LEACHED FROM ELASTOMERS

Biocide		Corrosion rate[a], mg/dm^2/day	
Code No.	Chemical Type	Polyurethane, Coating B[b], on Aluminum	Polysulfide, Sealant S-1[b], on Aluminum
GS-1	β-Nitrostyrene	0	0
GS-2	2-Nitroresorcinol	0	0
GS-3	8-Hydroxyquinoline salicylate	0	0
GS-4	Ethylene diacetate	82.01	0
GS-5	Alkyl quaternary acetate	126.37	4.10
GS-7	A morpholine derivative	14.10	(c)
GS-8	8-Hydroxyquinoline	2.80	0

(a) Corrosion rate after 28 days exposure in distilled water, milligrams/square decimeter/day.
(b) Contained 2 per cent biocide based on dry weight of composition.
(c) Sealant not compatible with biocide.

Biocidal Additives in a Polyurethane Coating

Ninety-two experimental compounds were evaluated. Of these, 65 were received from 16 commercial sources and 27 were added from Battelle stocks. Many were not identified by the supplier, as requested, and some obviously did not meet the above criteria because of the presence of heavy metals, sulfur, etc. The compounds screened are identified in Table 19, which also includes data on their solubility in fuel and water, compatibility with Coating B, and results obtained in exposure tests.

Twenty-seven of the compounds examined were not appreciably soluble in fuel or water and were compatible with Coating B. Thus, in these respects, they were suitable for evaluation. These compounds are noted in Table 19. In addition, 21 compounds with varying degrees of fuel and water solubility were evaluated in mixed-inoculum experiments.

Of the 48 compounds evaluated, nine have shown ability to prevent growth on the coating surface for at least 28 days. These were selected for further study. In early studies, it was found that a 2-phr* level of biocide in Coating B was not sufficient to provide extended protection for this coating. Therefore, 20 phr was selected as the biocide concentration in initial studies. A number of factors enter into the need for higher concentrations for protection. These include the biocidal effectiveness of the compounds, blocking of biocidal effect by the polyurethane, rate of biocide release from the coating, and fuel and water solubility of the compounds.

On the basis of data obtained to date, however, the nine compounds showing the greatest promise are AC-1, AC-3, L-1, L-3, NC-5, NC-6, O-1, UC-4, and WC-4. It should be noted, however, that L-1 and L-3 do not have the desired low solubility in fuel and water. The data reflect the relative effectiveness of the compounds in protecting Coating B, although direct comparison of biocide performance in experiments initiated at different times is not always conclusive.

* Phr - parts per hundred of resin coating solids.

TABLE 19. IDENTIFICATION AND EVALUATION OF BIOCIDES IN A POLYURETHANE COATING FOR ALUMINUM

Battelle Code No.[a]	Chemical Identification[b]	Solubility[c]		Compatibility With Two-Part Polyurethane Coating[d]	Comments[e]
		Fuel	Water		
AC-1	A thiocyanate	IS	IS	C	Promising
AC-2	A guanidine	S	IS	C	--
AC-3	An organic arsenical	PS	IS	C	Promising
BL-1	A metaborate	IS	IS	C	Not effective
BMI-1	Polycarbodiimide	IS	IS	C	Not effective
BMI-2	Paranitro benzonitrile	IS	IS	C	Not effective
BMI-3	2-nitro-2-ethyl-1,3-propanediol	IS	S	C	Not effective
BMI-4	X-cide, XC-370	IS	S	NC	--
BMI-5	2-nitro-1-butanol	IS	S	NC	--
BMI-6	2-nitro-2-methyl-1-propanol	S	S	C	Not effective
BMI-7	Sorbic acid	IS	PS	C	Ditto
BMI-8	Sodium o-phenylphenate $4H_2O$	IS	PS	NC	--
BMI-9	Sodium trichlorophenate	IS	S	NC	--
BMI-10	Chloro-o-phenylphenol	S	IS	C	Not effective
BMI-11	Phenyl mercury acetate	IS	S	NC	--
BMI-12	Sodium salt of mercaptobenzothiazole	IS	PS	NC	--
BMI-13	Dodecyl dimethyl benzyl ammonium cyclopentane carboxylate salt	S	S	NC	--
BMI-14	Sodium salt of dimethyl dithiocarbonic acid and of 2-mercapto-benzothiazole	IS	IS	NC	--
BMI-15	2,2'-methylene bis (4-chlorophenol) aqueous solution	IS	IS	NC	--
BMI-16	Pentachlorophenol	IS	IS	C	Not effective
BMI-17	Sodium propionate	IS	IS	C	Ditto
BMI-18	2-mercapto-benzothiazole	IS	IS	C	"
BMI-19	Sodium diethyldithiocarbonate	IS	S	NC	--
BMI-20	Sodium dimethyldithiocarbonate	S	S	NC	--
BMI-22	2,2'-methylene bis (4-chlorophenol), technical grade	IS	IS	C	Not effective
BMI-23	p-nitrophenol	IS	PS	C	Ditto
BMI-24	o-nitrophenol	S	PS	C	"
BMI-25	Salicylic acid	IS	PS	NC	--
BMI-26	Salicyl [illegible]ide	IS	IS	NC	--
BMI-27	2,3-dic[illegible]oro-1,4 naphthoquinone	PS	IS	C	Not effective
BMI-28	Potassium dichromate	IS	S	PC	Ditto
CCW-1	An organotin	S	IS	C	--
CCW-2	Ditto	S	IS	C	--
CCW-3		S	IS	C	Not effective
CCW-4	"	S	IS	C	Ditto
CCW-5	Not identified	IS	IS	C	"
CCW-6	A [illegible]ocarbamate	--	--	--	--
CCW-7	An organotin	S	S	C	Not effective
CCW-8	Ditto	PS	IS	C	Ditto
CSC-1	A morpholine	S	S	--	--
FO-1	Not identified	IS	IS	C	Not effective
FO-2	Not identified	IS	IS	C	Ditto
GS-1	*β*-nitrostyrene	S	IS	C	"
GS-2	*β*-nitroresorcinol	S	S	C	--
GS-3	8-hydroxyquinoline salicylate	S	S	C	--
GS-4	Ethylidene diacetate	S	S	C	--
GS-5	Alkyl quaternary acetate	PS	S	C	--

TABLE 19. (Continued)

Battelle Code No.[a]	Chemical Identification[b]	Solubility[c]		Compatibility With Two-Part Polyurethane Coating[d]	Comments[e]
		Fuel	Water		
GS-6	An organic borate	PS	S	NC	--
GS-7	A morpholine	S	S	C	Not effective
GS-8	8-hydroxyquinoline	S	PS	C	Ditto
GS-9	Nitrobenzene	S	S	NC	--
GS-10	A borane	PS	S	NC	--
HC-1	A saccharinate	IS	IS	NC	--
HC-2	Ditto	IS	IS	NC	--
HC-3	"	IS	S	C	Not effective
L-1	A pyridine	IS	S	C	Promising
L-2	A nitrile	S	IS	C	Not effective
L-3	A nitrile	S	IS	C	Partially effective
L-4	A furazan	S	IS	C	Not effective
L-5	A furazan	S	IS	C	Ditto
L-6	An oxiodinium	IS	IS	C	"
L-7	Ditto	IS	IS	C	"
L-8	A benzamide	IS	IS	C	"
L-9	An oxiodinium	IS	IS	C	"
MC-1	Cynuric acid	IS	IS	NC	--
NC-1	Not identified	S	IS	C	--
NC-2	Ditto	S	S	C	--
NC-3	"	S	IS	C	--
NC-4	"	S	IS	C	--
NC-5	"	IS	IS	C	Partially effective
NC-6	"	IS	IS	C	Ditto
NC-7	"	S	IS	C	--
NPD-1	"	S	IS	--	--
NPD-2	A phthalimide	IS	IS	C	Not effective
O-1	A thione	IS	IS	C	Promising
O-2	A thione	IS	IS	C	Not effective
O-3	Ditto	IS	IS	C	Ditto
O-4	"	IS	IS	C	"
O-5	"	IS	S	C	"
SC-1	(-nitrovinyl) toluene	S	IS	C	--
SC-2	-nitrostyrene	S	IS	C	--
SC-3	An acetoxy dioxane	S	S	C	--
SOC-1	An organic borate	IS	IS	C	Not effective
SOC-2	Not identified	S	IS	C	--
UC-1	Ditto	IS	IS	C	Not effective
UC-2	"	IS	IS	PC	--
UC-3	"	S	IS	PC	--
UC-4	"	S	IS	PC	Partially effective
WC	An acetonitrile	S	S	C	--
WC	Ditto	IS	IS	C	Not effective
WC-	"	PS	S	C	--
WC-4	"	IS	IS	C	Partially effective

Footnotes appear on following page.

Footnotes for Table 19

(a) Source of the biocides:

AC	- American Cyanamid Company	MC	- Monsanto Chemical Corporation
BL	- Buckman Laboratories	NC	- Nalco Chemical Company
BMI	- Battelle Memorial Institute	NPD	- Nuodex Products Company
CCW	- Carlisle Chemical Works, Incorporated	O	- Olin Matnieson Chemical Company
CSC	- Commercial Solvents Corporation	SC	- Scientific Chemical Incorporated
FO	- Fine Organics, Inc.	SOC	- Standard Oil Company (Ohio)
GS	- Gulf Research and Development Company	UC	- The Upjohn Company
HC	- R. M. Hollingshead Corporation	WC	- Wyandotte Chemicals Corporation
L	- Eli Lilly and Company		

(b) Compounds identified generically to protect proprietary rights of the various companies. Obviously, some compounds do not meet all of the biocide criteria, but in some cases compounds found to be insoluble in fuel and water were evaluated.

(c) Determined visually at room temperature over a period of 14 days. Approximately 50 mg of the candidate compound was placed in a test tube containing 10 ml of deionized water or Searsport JP-4 fuel. S = soluble, PS = partly soluble, and IS = insoluble.

(d) C = compatible, NC = not compatible, and PC = partially compatible (minor adverse effects).

(e) Comments pertain to addition of biocides to Coating B. The coating was then applied to aluminum rods, cured, and exposed to the standard mixed microbial inoculum. "Not effective" means protection from microbial attachment for less than 28 days at 2-20 phr. The "--" notation means not evaluated.

In addition to the arbitrarily prescribed and previously mentioned criteria for biocidal material, there are several other important factors that enter into the selection of a protective additive for coating materials. These include (1) maximum protection possible based on both degree of growth and duration of effectiveness, (2) effectiveness at low level, (3) ability to resist growth in changing media (simulating operational conditions where fuel might be added frequently), and (4) effect of the additive on cure and compatibility with the coating. Item (4) is covered in Table 19 but it can be stated here that most of the preferred biocides have little observable effect on a two-part polyurethane coating.

Data obtained on the nine selected compounds are presented in Table 20. On the basis of minimum times for the appearance of profuse growth in fixed media and the degree of growth in changing media, Biocides AC-1 and WC-4 appear best at a high concentration level. Compound WC-4 is preferable, based on minimum effective levels required in fixed media. Based on the degree of growth occurring in changing media, Compound AC-1 appears to be effective at a low concentration level.

It is quite obvious from the table that additional data are needed to confirm conclusions reached on these nine materials. Biocides designated AC-1, AC-3, L-1, L-3, and O-1 were received rather late in the program. Thus, it was not possible to complete all of the desired investigations of these materials. If all of the data were available, it would be possible to make firmer and more discriminating decisions. It is possible that the biocide coded AC-3 may also be an effective material. In this same connection Biocide L-1 appears to be quite effective with respect to the time for growth to appear as well as on the minimum level required to prevent growth in both fixed and changing media. It should be pointed out that L-1 is soluble in water and virtually insoluble in fuel. This may be an advantage. From the data available, the AC-1 and WC-4 biocides at 5 and 10 phr, respectively, appear to offer the greatest promise at the present time. The preferred AC-1 (an organic thiocyanate), at a 5-phr level, has remained effective through the 40-day period of observation, even when the exposure medium of fuel, water, and microorganisms was replaced every 10 days. Laboratory media which did not contain high concentrations of anti-icer were heavily inoculated initially. Thus, experimental conditions represent an acceleration of those encountered operationally.

Further work with these biocides – especially on long-term studies – should be conducted before firm recommendations and detailed instructions for their use can be made. It is also quite possible that improved performance can be obtained by the use of combinations of selected biocides.

Effect on Physical Properties of Coating. For many biocides, additions to Coating B must be made in concentrations up to 20 phr in order to control microbial growth. Thus, some question naturally arises concerning the effect of such large additions on the properties of the coating. To answer this, one of the more effective biocides found during the study (WC-4) was incorporated in the two-part polyurethane (Coating B). The biocide was added by ball-milling it into the B component of this coating in order to obtain a uniform dispersion. The coating was spray applied to aluminum panels to a dry-film thickness of 2 mils. The product was then screened for its performance in some of the more rigid tests selected from MIL-C-27725A.

Results of the evaluation are shown in Table 21. In general, the coating with biocide performed as well as the unmodified coating, passing all but two tests from MIL-C-27725A. In the iron chloride resistance test (3.3.13) the modified coating had

TABLE 20. PERFORMANCE OF PROMISING BIOCIDES

Biocide Code Number	Minimum Time (Days) for Appearance of Profuse Microbial Growth, 20 Phr Biocide, Fixed Media		Degree of Growth on Coating, 20 Phr Biocide, Changing Media[b]	Minimum Effective Biocide Level (Time in Days for Profuse Growth to Appear on Coating), Fixed Media			Degree of Growth on Coating After 40 Days, Changing Media[c]		
	In Media	On Coating		5 Phr	10 Phr	15 Phr	5 Phr	10 Phr	15 Phr
AC-1	-	240	Very slight	-	-	-	None	None	None
AC-3	3	230[a]	None	-	-	-	-	-	-
L-1	90[a]	230[a]	None	-	-	-	Profuse	None	None
L-3	90[a]	230[a]	Profuse	-	-	-	-	-	-
NC-5	270[a]	270[a]	Ditto	7	14	180	-	-	-
NC-6	270	270[a]	"	7	7	28	-	-	-
O-1	10	30[a]	None	-	-	-	-	-	-
UC-4	30	60	Profuse	60	60	60	-	-	-
WC-4	180[a]	180[a]	Slight	28	180[a]	180[a]	-	-	-
No biocide	2-3	5-7	Profuse		7 days			Profuse	

(a) No growth at time indicated.
(b) Media changed at 10-day intervals — 3 exposures (30 days).
(c) Media changes at 10-day intervals — 4 exposures (40 days).
[Growth in the medium invariably occurred in the Bushnell-Haas and at the fuel-water interface during each 10-day interval in changing media systems (b and c)].

TABLE 21. PERFORMANCE OF POLYURETHANE COATING CONTAINING 20 PHR OF BIOCIDE[a]

MIL-C-27725A Test Procedure			
No.	Title	Requirement	Results
3.3.1	Application properties	Smooth, uniform film	Yes
3.3.2	Color	Translucent coating	Yes
3.3.3	Weight per gallon	Within ±5% of qualification value	10 lb
3.3.4	Nonvolatile content	Not less than 35%	56%
3.3.5	Viscosity	10-20 seconds	16 seconds
3.3.6	Application life	8 hours	2 hours[b]
3.3.7	Drying time	4-hour tack-free time	3-3.5 hours
3.3.8	Cure time	14 days	Yes
3.3.9	Resistance to water	No failure in scored coating after 30 days at 140°F	OK
3.3.10	Resistance to salt water and fuel	Ditto	OK
3.3.12	Resistance to hydraulic fluid	No failure in scored coating after 14 days at 180°F	OK
3.3.15	Low-temperature flexibility	No cracking when bent over jig at -65°F	OK
3.3.19	Repairability	Good adhesion of repair coat	OK
3.3.20	Resistance to simulated microbial by-products	Good adhesion after exposure for 5 days to salt water-acetic acid solution at 140°F	
3.3.13	Resistance to iron chloride	1-megohm resistance after 10 days at 140°F in iron chloride	8000 ohms[c]
3.3.14	Fuel contamination	Nonvolatile extractibles less than 20 milligrams	OK
3.3.16.1	Compatibility of sealant with coating material	Minimum sealant-to-coating peel strength of 20 lb/in. after 140°F, 7-day exposure to salt water/fuel	15 lb/in.

(a) Biocide WC-4 (a piperazine derivative), Wyandotte Chemical Company.

(b) Coating was still usable after 2 hours, but was grainy and obviously changed from its freshly mixed condition. A control coating which contained no biocide behaved in a similar manner. Thus, it did not appear that the biocide greatly affected application life of the coating.

(c) A resistance value of 8000 ohms was also obtained with an unexposed coating containing biocide.

resistance values of only 8000 ohms. This is below the requirement, but does not necessarily indicate poor corrosion protection. Likewise in 3.3.16.1, "Compatibility of Sealing Compound to Coating Material", the peel strength of a polysulfide sealant applied over the coating was less than the specified minimum of 20 pounds/in. However, the sealant failed in cohesion, so that up to 15 pounds/in. there was no indication of the biocide affecting adhesion. It is concluded that large quantities of biocide may be added to Coating B without adversely affecting its performance but that properties of specific coating-biocide mixtures should be checked in critical qualification tests. As long as there is no reaction between the biocide and coating components, it may be assumed that the biocide is acting as a filler in the coating. Thus, it is felt that the results of this study are applicable to other biocides within this important limitation.

Biocidal Additives in an Epoxy Coating

Four compounds (AC-1, NC-5, NC-6, and WC-4) were evaluated after incorporation in Coating N at 20 phr and application on steel rods. This epoxy-based coating is used primarily in steel fuel-storage tanks. The evaluation consisted of the standard single-exposure, mixed-inoculum system described previously. After 8 months of exposure, all of the compounds effectively prevented growth in the medium and attachment of growth to the coating. AC-1 appears to be the most promising of the compounds evaluated in the representative steel-tank coating studied. These results are encouraging as far as they go. Further work should be done, particularly with exposures in which the medium is periodically replaced. This would indicate the effectiveness of residual amounts of biocide in the coating and also give an indication of the degree of extraction of biocide from the coating.

Compatibility of Fuel-System Elastomers and Coatings With Fuel Biocides

The Air Force has carried out studies involving the direct addition of biocides to fuel to control the growth of microorganisms. The amount of biocide added is only about 0.1 per cent. However, if a biocide is soluble to any degree in water, it may become concentrated in any water present in the fuel through a partitioning effect. Whatever its concentration in the fuel or water, the possibility exists that a biocide might have a deleterious effect on rubber seals, gaskets, tubing, or other elastomeric parts in the fuel system. Therefore, a study was carried out to determine the compatibility of elastomeric components with selected biocides.

The Air Force Aero Propulsion Laboratory supplied three biocides identified as B-308, β-nitrostyrene, and arsenosobenzene. These were added to JP-4 fuel in specified amounts to form exposure systems representative of "protected fuel" for military aircraft. In addition Biocide B-308, which is somewhat soluble in water, was made up as a 6 per cent water solution to simulate conditions in the bottom of wing tanks and sump areas. The arsenosobenzene was received rather late in the program and, because of this, was not evaluated as completely as the other two biocides. The exposure systems used in this study were as follows:

(1) JP-4 fuel containing 0.1 per cent anti-icer.

(2) JP-4 fuel, 0.1 per cent anti-icer, and 0.02 per cent B-308.

(3) Distilled water.

(4) Distilled water containing 6 per cent B-308.

(5) Fuel-water mixture consisting of equal parts of (2) and (4).

(6) JP-4 fuel, 0.1 per cent anti-icer, and 0.1 per cent β-nitrostyrene.

(7) JP-4 fuel, 0.1 per cent anti-icer, and 0.02 per cent arsenosobenzene.

Elastomers evaluated for compatibility with the biocides included nitrile, neoprene, Viton A, silicone, and polyurethane types. Since these elastomers are used primarily as seals, they are seldom exposed to water which has separated from fuel. Therefore, these elastomers were exposed only to fuel solutions of the biocides. Exposure was for 3 days at 75°F. Tensile strength, elongation, hardness, and volume change were determined immediately after removal of specimens from the fuel. Results are shown in Table 22.

Other fuel-system components evaluated in the compatibility study included two bladder cell materials, a repair cement, four polysulfide sealants, and 10 coatings qualified for use on either aluminum or steel. The bladder cell materials and repair cement were exposed for 7 days at 140°F. The coatings and sealants were exposed for 70 days at 140°F. The ply strength of one of the bladder cell materials was weakened considerably in water. It was planned to repeat this exposure using new material. However, the project terminated before a new sample of this bladder material was received. Results of these exposures are summarized in Tables 23, 24, and 25.

Biocide B-308. Biocide B-308 in fuel had no identifiable effect on any of the elastomers after 3 days of exposure at 75°F. Similarly, fuel solutions of this biocide had no effect on the polyurethane or nitrile rubber bladder cell materials, or on the repair cement, after 7 days at 140°F. In a more severe exposure (70 days at 140°F) the Buna N coating was softened more in fuel containing B-308 than in a biocide-free fuel. None of the other coatings was affected as far as could be determined by means of pencil-hardness measurements. B-308 may have reduced the strength of three of the four polysulfide sealants. This is reflected by a reduction in the average peel strength of these materials. In addition, one sealant exposed to fuel containing B-308 lost adhesion to Iridited aluminum.

Most materials exposed to water had a significant loss in strength. Therefore, it is difficult to determine whether B-308 in the water had any further effect. Of the two bladder cell materials, the polyurethane was affected more by water exposure. The addition of B-308 to the water appeared not to have caused any further change. The same can be said for the repair cement. There is an indication that B-308 in water has a possible softening effect on the polyurethane coating. Similarly, B-308 may have reduced adhesion of Sealant S-1 to aluminum. Since the results were observed in both the coating and sealant applied over one type of aluminum treatment but not the other, the results may not be conclusive.

β-Nitrostyrene. The second biocide, β-nitrostyrene, was evaluated in fuel solution only, since its solubility in water is quite low. This biocide appears to have caused a slight softening of the nitrile rubber samples exposed for 3 days at 75°F. The loss in tensile strength on which this is based is within the limits of experimental error, and

TABLE 22. RESISTANCE OF ELASTOMERS TO FUEL CONTAINING BIOCIDES

Exposure: 3 Days at 75°F

Elastomer	Test	Property Value After Exposure in Indicated System[a]				
		Unexposed	JP-4 Fuel[4]	Fuel + 0.02% B-308	Fuel + 0.1% β-nitrostyrene	Fuel + 0.02% arsenosobenzene
Viton A (MIL-R-25897)	Tensile strength, psi	2580	2110	2210	2050	2200
	Elongation, %	420	410	410	410	350
	Hardness, Shore A	64	61	62	58	63
	Volume change, %	0	-0.16	+0.6	0	--
Silicone (MIL-R-25988)	Tensile strength, psi	980	500	470	450	420
	Elongation, %	200	130	130	150	120
	Hardness, Shore A	48	46	45	45	47
	Volume change, %	0	-1.0	-0.6	-0.5	--
Buna N (MIL-P-5315)	Tensile strength psi	1970	1560	1510	1300	600
	Elongation, %	290	220	210	210	60
	Hardness, Shore A	57	52	50	47	61
	Volume change, %	0	-1.1	-1.0	-1.2	--
Polyurethane	Tensile strength, psi	3250	2850	3150	2880	2700
	Elongation, %	500	450	450	480	450
	Hardness, Shore A	60	57	58	55	54
	Volume change, %	0	-3.0	-1.9	-2.2	--
Neoprene (R&S stock)	Tensile strength, psi	3450	1610	1500	1600	1600
	Elongation, %	260	150	130	160	180
	Hardness, Shore A	72	55	56	55	55
	Volume change, %	--	+49.8	+50.7	+52	--
Marman Clamp (Pacific Moulded Products Co.)	Tensile strength, psi	1740	400	450	400	--
	Elongation, %	270	100	100	100	--
	Hardness, Shore A	62	40	40	42	--
	Volume Change, %	--	+79	+83	+94	--
Marman Clamp (Kirkhill Rubber Co.)	Tensile strength, psi	--	700	500	500	--
	Elongation, %	--	120	80	90	--
	Hardness, Shore A	--	57	53	53	--
	Volume change, %	--	+62	+62	+65	--
Marman Clamp (Plastic and Rubber Products Co.)	Tensile strength, psi	2130	400	400	530	--
	Elongation, %	500	100	100	100	--
	Hardness, Shore A	68	45	47	43	--
	Volume change, %	--	+97	+100	+97	--

(a) Property values are the average of three specimens.

(b) All fuel, including that with biocides, contained 0.1% anti-icer.

TABLE 23. COMPATIBILITY OF BLADDER-CELL MATERIALS AND A REPAIR CEMENT WITH FUEL BIOCIDES AFTER 7 DAYS AT 140°F

Material	Test	Unexposed	Property Value After Exposure in Indicated System (1) JP-4 Fuel[a]	(2) JP-4 + 0.02% B-308	(3) Dist. Water	(4) Water + 6% B-308	(5) 1:1 Mixture of (2) and (4)	(6) JP-4 + 0.1% β-Nitrostyrene
Bladder Cell A (polyurethane)	Tensile strength, psi	7890	6330	7660	5110	4330	4000	6110
	Elongation, %	470	470	480	480	500	480	480
Bladder Cell B (Fabric-reinforced nitrile rubber)	Tensile strength, psi	8610	8490	5970	5910	5620	5510	6370
	Elongation, %	40	40	30	30	30	30	30
	Seam peel strength, lb/in.	13	13	13	5[b]	6.5[b]	8.5	11
MIL-A-9117 Repair cement with Bladder Cell A	Peel strength, lb/in.	4.1	1.5	1.4	1.4	0.5	1.7	0.9
MIL-A-9117 Repair cement with Bladder Cell B	Peel strength, lb/in.	5.0[b]	1.8	2.1	1.5	1.3	2.2	1.1

(a) All JP-4 Fuel, including that with biocides, contained 0.1% anti-icer.

(b) Rubber separated from fabric during peeling; therefore, seam strength of bladder cell material and strength of repair cement were not actually determined.

TABLE 24. COMPATIBILITY OF FUEL BIOCIDES WITH COATINGS

Exposure: 70 Days at 140°F

Coating[a] Code	Coating[a] Type	Metal Substrate	Pencil Hardness of Unaged Controls	Change From Initial Pencil Hardness Value After Exposure in Indicated System[b] (1) JP-4 Fuel[c]	(2) JP-4 + 0.02% B-308	(3) Dist. Water	(4) Water + 6% B-308	(5) 1:1 Mixture of (2) and (4)	(6) JP-4 + 0.1% β-Nitrostyrene	(7) JP-4 + 0.02% Arsenosobenzene[d]
A	Buna N (MIL-S-4383B)	Anodized Al	2H	0	-4	-9	-8	-9	-2	0
		Iridited Al	3H	0	-4	-5	-8	-6	-4	0
B	Polyurethane (MIL-C-27725A)	Anodized Al	2H	0	0	0	-5	-4	+1	0
		Iridited Al	2H	0	+4	+4	+3	-2	+1	+4
P	Epoxy (BMS 10-39	Anodized Al	H	-3	-3	-2	-3 Blisters	-8 Blisters	-2	-1
		Iridited Al	H	-3	-3	-2	-2	-2	-2	-1
M	Epoxy (BMS 10-39)	Anodized Al	2H	+4	+4	-4 Blisters	-3 Blisters	-3 Blisters	+3	+2
		Iridited Al	H	+5	+5	-3 Blisters	-1	-2	+4	+2
L	Inorganic zinc (MIL-C-4556B)	Steel	H	-6	-6	Loss of adhesion	Blisters & Loss of adhesion	Loss of adhesion	-3	-1
N	Epoxy (MIL-C-4556B)	Steel	2H	-4	-4	-4	-4 Blisters	-3	-3	-2
V	Epoxy (MIL-C-4556B)	Steel	3H	+1	+2	-4 Blisters	-4 Stight blistering	-4 Blisters	+2	+2
W	Epoxy (MIL-C-4556B)	Steel	3H	-5	-5	-4	-4	-10	-4	+2
U	Epoxy (MIL-C-4556B)	Steel	HB	-1	-1	-2 Blisters	-3 Blisters	0 Blisters	0	0
I	Furan	Steel	2B	+1	+1	+1 Blisters	+1	+2	+2	+4

(a) Coatings were applied to aluminum sheet according to procedures in MIL-C-27725A, MIL-C-4556B, and MIL-S-4383B.

(b) Numbers indicate increase or decrease in hardness and number of grades change from the initial pencil-hardness reading. Grades are based on the following scale: 6B, 5B, 4B, 3B, 2B, B, HB, F, H, 2H, 3H, 4H, 5H, 6H.

(c) All JP-4 fuel contained 0.1% anti-icer.

(d) Arsenosobenzene was received late in the program. Therefore specimens were exposed to this biocide for only 41 days.

TABLE 25. COMPATIBILITY OF FUEL BIOCIDES WITH POLYSULFIDE SEALANTS

Exposure: 70 Days at 140°F

Sealant[a] Code	Sealant[a] Type	Metal Substrate	Peel Strength, lb/in. Unaged Controls	(1) JP-4 Fuel[b]	(2) JP-4 + 0.02% B-308	(3) Distilled Water	(4) Water + 6% B-308	(5) 1:1 Mixture of (2) and (4) Water Phase	(5) 1:1 Mixture of (2) and (4) Fuel Phase	(6) JP-4 + 0.1% β-Nitrostyrene
S-1	Polysulfide	Anodized Al	18.5	20.5	15	18.3	1	1	24	21.5
		Iridited Al	24	25.2	17	3	1	1	28	24
S-2	Polysulfide	Anodized Al	17	13.8	16.7	1.5	1	1	20	18
		Iridited Al	21	18.7	15	0.5	1	1	4.5	22
S-3	Polysulfide	Anodized Al	21	23	20	0.5	1	1.7	27	25.5
		Iridited Al	17	27	23	0.5	1	2	30	29
S-4	Polysulfide	Anodized Al	24	24	20.8	18	12.3	1.2	24.5	23
		Iridited Al	25	18	2	22	20	20	25	18

(a) Sealants were applied to aluminum sheet for testing according to procedures in MIL-S-7502C, and MIL-S-8802C.

(b) All fuel, including that with biocides, contained 0.1% anti-icer.

variations of this magnitude are not uncommon in rubber testing. Therefore, this observation may not indicate significant incompatibility between nitrile rubber and β-nitrostyrene. This could be determined using a greater number of specimens in a more severe exposure. The β-nitrostyrene caused no significant change in the measured properties of the bladder cell materials, repair cement, coatings, or sealants.

Arsenosobenzene. Arsenosobenzene was evaluated only in fuel solutions. Because it was received late in the program, coating exposure was limited to 41 days rather than the full 70-day period. This biocide had no apparent effect on any of the coatings. However, arsenosobenzene caused a significant loss in strength and elongation of the nitrile rubber after a relatively mild exposure (3 days at 75°F). There is no obvious reason why it should have affected the nitrile rubber and not the Buna N coating under much more severe exposure conditions. However, it is possible that pencil-hardness measurements were not capable of detecting strength changes in the coating film.

In summary, water was responsible for the most significant change in properties of any of the materials evaluated. Most elastomers are apparently not affected by the three biocides, but arsenosobenzene should be checked for its compatibility with specific nitrile rubber formulations before use in a fuel system. β-nitrostyrene caused no significant effect except for possible softening of the Buna N coating. B-308 in water was involved in suspected incompatibility with a wide variety of materials. However, because of the overriding effect of the water, no clear conclusion can be reached.

Corrosion of Aluminum

Factors Affecting Corrosion

Attempts have been made to answer certain questions concerning corrosion. The project engineer asked that these be explored on a limited scale to shed additional light on the interrelationship between the action of microorganisms and certain water bottoms, on aluminum. These studies were carried out in two principal areas: the first concerned the effect of impurities in the water on the corrosion of aluminum and the second the corrosiveness of consolidated sump samples taken from SAC planes.

The purpose of the first study was to determine whether the ion strength of the water used in the laboratory corrosion studies had any effect on the rate of corrosion of aluminum. There had been some indication from other work that aluminum specimens corroded more rapidly in the presence of deionized water than in distilled water. To investigate this, anodized and Iridited aluminum rods were exposed in both sterile and inoculated 1:1 fuel/water mixtures. The water portion consisted of (1) standard Bushnell-Haas mineral salts, (2) Kereluk's No. 3 medium (nonbuffered mineral salts), (3) deionized water from Battelle, and (4) deionized water from The Ohio State University. The protein content of the latter was very low.

The exposure systems were maintained at 86°F in an incubator under constant rotation. Various patches of dark stain appeared on several of the aluminum specimens. However, corrosion, as evidenced by pits in the aluminum, was not observed in any instance. After 9 months' exposure, no evidence of pitting was found. It was concluded that the water samples used in the study were not corrosive to aluminum and that it was not possible to tell, on the basis of the experiment, whether the type of water has any

measurable effect on the rate of corrosion. Since all of the water portions were low in heavy metal-ion content and contained no chlorides, it can be said that little corrosion would be expected. The results also indicate that the mixed microbial inoculum consisting of Hormodendron cladosporoides, Cladosporium resinae, and three pseudomonads was not corrosive to anodized or Iridited aluminum under the conditions of this experiment.

Sump Samples From Various SAC Bases

Perhaps of greater importance was the study of the corrosiveness of water drained from sumps of SAC planes (B-52s and KC-135s). These specimens represented water bottoms collected at various Air Force bases by RTD personnel. The sump samples were checked at Battelle for microbial contamination and also for corrosiveness to 7075-T6 aluminum. Despite repeated attempts, no evidence of microorganisms was found by a standard plate-count technique in any of the sumps, as received. This was undoubtedly due to the high anti-icer content (up to 37 per cent) in the sumps.

For the corrosion study, the specimens were placed on glass supports in flat Pyrex dishes and covered with a loose-fitting glass plate. Results of 105 days of exposure of 5 samples at 80°F are given in Table 26. It can be seen that all sump samples

TABLE 26. CORROSION DATA FOR 7075-T6 ALUMINUM SPECIMENS EXPOSED FOR 105 DAYS TO SUMP WATER AT 80°F

Sump-Water Sample	Specimen	General Corrosion Rate, mils/year[a]	Max Pit Depth, mils	Calculated Max Pit Depth, mils/year	Appearance
WPAFB,	Untreated sheet	0.61	8.0	27.8	General corrosion, 4 pits
11/29/63	Untreated sheet	0.70	8.0	27.8	Ditto
WPAFB,	Untreated sheet	0.11	--	--	General corrosion, no pits
1963	Untreated sheet	0.13	2.5	8.7	General corrosion, 1 pit
composite	Untreated rod	0.14	8.0	27.8	General corrosion, 2 pits
	Anodized rod	0.03	--	--	Dark golden stain, no pits
	Iridited rod	0.03	10.0	34.7	Localized attack, 5 pits, 1 blister, max height 10 mils
WPAFB,	Untreated sheet	0.24	--	--	General corrosion, no pits
12/3/63	Untreated sheet	0.26	4.0	13.9	General corrosion, 1 pit
(Contained	Untreated rod	0.30	16.0	55.5	General corrosion, 5 pits
blue dye)	Anodized rod	+0.05	--	--	Dark golden stain, no pits
	Iridited rod	0.19	16.5	57.3	Localized attack, 4 pits
Ramey AFB,	Untreated sheet	0.10	--	--	General corrosion, no pits
1/64	Untreated sheet	0.09	--	--	Ditto
	Untreated rod	+0.07	5.0	17.4	General corrosion, 2 pits
	Anodized rod	+0.02	--	--	Faint golden stain, no pits
	Iridited rod	+0.05	17.0[b]	59.0[b]	Localized attack, 3 pits
Eglin AFB,	Untreated sheet	0.09	6.5	22.6	General corrosion, 1 pit
9/63	Untreated sheet	0.08	1.0	3.5	Ditto
	Untreated rod	0.06	2.0	6.9	General corrosion, 2 pits
	Anodized rod	0.00	--	--	Dark golden stain, no pits
	Iridited rod	0.03	8.0	27.8	Localized attack, 4 pits

(a) "+" means rate based on weight gain. $Al_2O_3 \cdot 3H_2O$; all others based on weight loss.
(b) Measured from metallographic section.

are capable of causing corrosion. However, corrosive action was most general in the case of nontreated (uncoated) aluminum, although the Iridited aluminum specimens also developed pits. No corrosion was evident on the anodized specimens. The weight gain reported for the anodized specimens represents the formation of $Al_2O_3 \cdot 3H_2O$.

Although the initial concentration of anti-icing additive was reported to be relatively high, this additive was lost by evaporation during the exposure period. As the concentration of anti-icer dropped, microorganisms were observed to be growing in the water. Twenty-two isolates – 19 bacteria and 3 fungi – were taken from these particular sumps. Some of the bacteria were pseudomonads, but no fungi of the Cladosporium-Hormodendron groupings were found. None is believed to be involved in the corrosion of the aluminum specimens.

An additional set of water bottoms was supplied to Battelle at a later date. These were also used as exposure solutions for 7075-T6 aluminum rods. Exposures were carried out at 80°F, as above. The water solutions were overlaid with JP-4 fuel in order to reduce evaporation of the anti-icing additive. Corrosion rates for this second set of sump samples are given in Table 27. These results parallel the first ones, with corrosion in some cases being even greater than for the first set. A rather heavy accumulation of material appeared on the surface of several of the aluminum rods exposed in this experiment. This is illustrated in Figure 31. Since the anti-icing additive concentration was in the range of 35 per cent, it was doubtful that the surface accumulation represented microbial growth. Efforts to isolate microorganisms were negative, as were attempts to characterize microscopically, the material removed from the rods. A test for protein was also negative. Thus, it was concluded that the surface deposit was composed of hydrated aluminum oxides rather than microbial growth. Similar corrosion deposits have been observed on aluminum exposed to fresh water. Photographs of corroded rods and microphotographs showing the extent of pit corrosion are presented in Figures 31 through 37.

Correlation Between Sump Contaminants and Corrosivity. The various sump samples and composites were analyzed by another USAF contractor, and the analyses are presented in Table 28. Data on all components and possible contaminants were not available for every sump specimen. For example, the anti-icer content of samples from Barksdale, Bergstrom, and K. I. Sawyer Air Force Bases were not available for inclusion in this report. In other cases where data were limited, the information has been omitted. This includes analyses for boron, bismuth, cobalt, phosphorus, zirconium, and platinum. Platinum determinations apparently were made in only a limited number of instances, probably to determine whether the specimen had been contaminated by ashing in a platinum crucible. Also, two Wright-Patterson samples showed the absence or only trace quantities of certain elements. Naturally, conclusions could not be drawn from these results.

The first three sumps recorded in Table 27 represent samples received initially, and to which aluminum specimens were exposed for 105 days. The following seven specimens are from other Air Force bases and were received at a later date. Aluminum corrosion samples were exposed to this latter group of sumps for 42 days. The sump samples were ranked according to corrosivity on the basis of general corrosion rate in mils per year (for Iridited aluminum) and of anodal pitting rate in mils per year. Table 29 lists these results. In each case the sample at the top of the column is the most corrosive of the groups.

TABLE 27. CORROSION DATA FOR 7075-T6 ALUMINUM SPECIMENS EXPOSED FOR 42 DAYS TO SUMP WATER AT 80°F

Sump-Water Sample	Specimen	General Corrosion Rate, mils/year[a]	Max Pit Depth, mils	Calculated Max Pit Depth, mils/year	Appearance
Barksdale AFB, 3/64	Untreated sheet	0.16	6.0	52.0	Two areas of general corrosion, 2 pits
	Untreated rod	0.16	8.0	69.4	A few areas of general corrosion, several pits
	Anodized rod	+0.04	--	--	No change in appearance, no pits
	Iridited rod	0.17	4.0	34.7	Localized attack, 5 pits
Bergstrom AFB, 4/1/64	Untreated sheet	0.42	2.0	17.3	General corrosion, 3 pits
	Untreated rod	1.08	8.0	69.4	General corrosion, 10 pits
	Anodized rod	+0.08	--	--	Light golden stain, no pits
	Iridited rod	0.73	25[b]	216.8[b]	Heavy localized attack, 20 blisters, max height 14 mils
K. I. Sawyer AFB, 3/64	Untreated sheet	0.10	--	--	One area of general corrosion no pits
	Untreated rod	0.19	4	34.7	2 areas of general corrosion, 1 pit
	Anodized rod	+0.03	--	--	No change in appearance, no pits
	Iridited rod	0.18	--	--	Only blunt end attacked, no pits
Loring AFB, 3/64	Untreated sheet	0.07	1.0	8.7	General corrosion, 1 pit
	Untreated rod	1.50	7.0	60.7	General corrosion, many pits
	Anodized rod	+0.03	--	--	No change in appearance, no pits
	Iridited rod	0.56	8.0	69.4	Localized attack, many pits
Columbus AFB, 3/26/64	Untreated sheet	0.08	3.0	26.0	General corrosion, 2 pits
	Untreated rod	0.10	4.0	34.7	General corrosion, 3 pits
	Anodized rod	+0.04	--	--	Light golden stain, no pits
	Iridited rod	0.06	5.0	43.4	Localized attack, 3 pits
Biggs AFB	Untreated sheet	0.09	3.5	30.3	Heavy general corrosion, 3 pits
	Untreated rod	1.33	8.0	69.4	Heavy general corrosion, 4 pits
	Anodized rod	+0.10	--	--	Light golden stain, no pits
	Iridited rod	0.95	14.0	121.4	Localized attack, 25 pits
Homestead AFB, 3/1/64	Untreated sheet	0.48	--	--	Light general corrosion, no pits
	Untreated rod	0.73	12.0	104.0	Light general corrosion, 4 pits
	Anodized rod	+0.03	--	--	No change in appearance, no pits
	Iridited rod	0.38	16.0	138.7	Localized attack, 13 pits

(a) "+" means rate based on weight gain, $Al_2O_3 \cdot 3H_2O$; all others based on weight loss.

(b) Measured from metallographic section.

TABLE 28. RESULTS OF ANALYSES OF AIRCRAFT FUEL-TANK

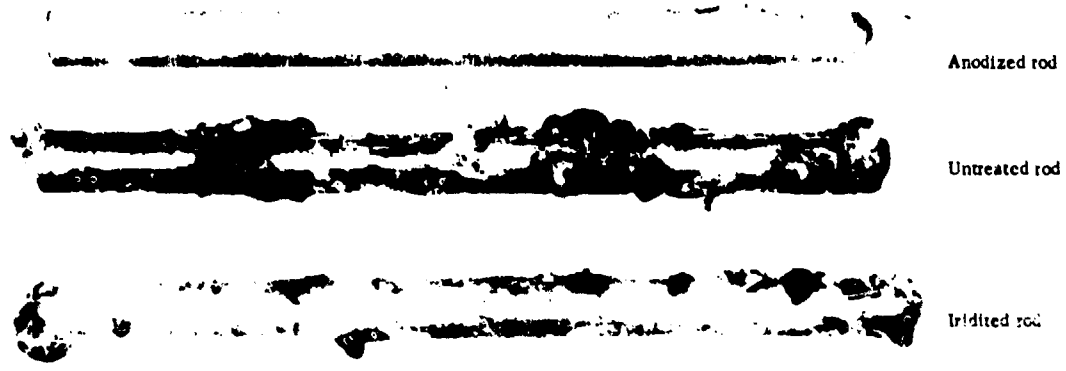
Sample Source	Date	Methyl Cellosolve Content vol/vol %	Glycerol Content, wt/vol %	Chloride, ppm	pH	Ag	Al	Ca	Cd	Cr	Cu
WPAFB	1963	--	--	--	--	0.04	15.8	3.4	2.4	0.40	0.32
Composite		27.3	0.67	119	5.38	<0.01	0.1	40.0	50.0	0.08	0.8
Ramey AFB	1/64	--	--	--	--	0.06	7.3	5.8	5.8	0.29	0.29
		27.0	0.095	124	6.95	ND	ND	20.0	0.7	0.2	0.6
Eglin AFB	9/63	--	--	--	--	0.01	16.1	10.1	1.0	0.14	0.20
		22.0	0.17	10	5.50	ND	0.3	20.0	20.0	0.05	0.6
Barksdale AFB	3/64]	--	--	--	--	0.001	11.9	2.4	0.95	0.48	0.60
		--	0.452	30	5.70	--	0.7	60.0	10.0	0.3	3.0
Bergstrom AFB	5/1/64	--	--	--	--	0.04	25.4	3.4	2.5	0.51	1.7
		--	3.07	909	5.01	--	8.0	--	>1000	0.08	5.0
K. I. Sawyer AFB	3/64	--	--	--	--	0.004	1.4	5.7	0.99	0.10	5.7
		--	0.140	161	5.62	--	0.3	20.0	2.0	0.10	12.0
Loring AFB	3/64	--	-	--	--	0.006	4.6	18.4	0.92	0.07	0.55
		37.0	0.27	286	4.50	--	0.03	8.0	2.0	--	1.0
Columbus AFB	3/26/64	--	--	--	--	0.006	24	3.6	3.6	0.12	0.30
		29.0	0.39	<5	5.00	--	0.03	20.0	30.0	<0.1	0.1
Biggs AFB	--	--	--	--	--	0.009	8.5	5.1	0.85	0.51	0.26
		37.0	0.43	92	4.70	--	5.0	80.0	100.0	0.1	1.0
Homestead AFB	/64	--	--	--	--	--	4.3	11.5	--	0.15	0.58
		19.0	0.06	56	5.50	--	0.03	20.0	5.0	<0.1	0.6

(a) Where two lines of data are shown, the top line refers to emission spectra semiquantitative analysis of ash of filter residue recorded as per cent. The second line is a chemical analysis of the filtrate and is recorded as ppm. The first four columns following the date represent analysis of the total unfiltered sump.

(b) The ranking of corrosivity of various sumps is based on the general corrosion rate (mils/yr) of iridited aluminum specimens exposed to the sump samples. Only the second group where more analytical information is available has been rated.

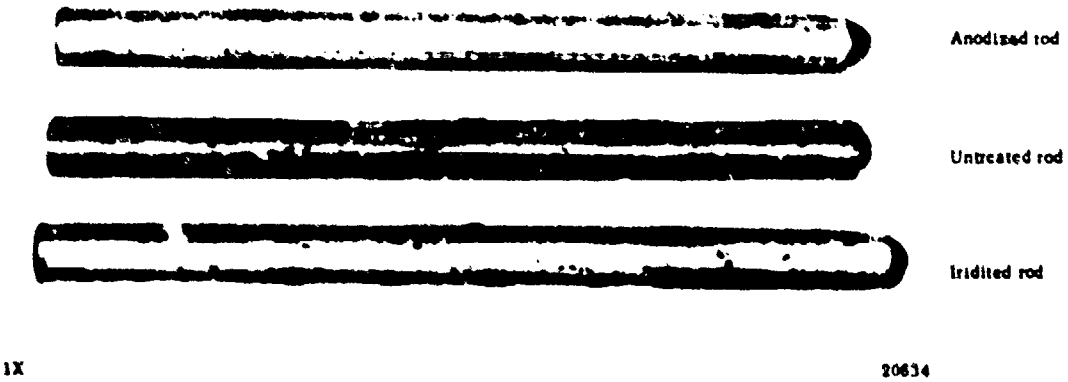
SUMP SAMPLES FROM VARIOUS AIR FORCE BASES[a]

Fe	Mg	Mn	Mo	Na	Ni	Pb	Si	Sn	Ti	Zn	Ash, grams	Corrosion Rank[b]
19.8	3.2	0.16	0.16	11.9	0.08	2.0	7.9	0.12	0.12	0.40	--	--
0.4	7.0	1.0	--	30.0	0.1	0.02	0.6	--	--	3.0	--	--
5.8	2.2	0.36	0.58	21.8	0.15	0.58	7.3	0.07	0.36	0.58	--	--
0.08	50.0	0.3	--	100.0	ND	ND	0.6	--	--	ND	--	--
2.0	2.0	0.60	0.10	16.1	0.10	0.60	8.0	<0.20	0.20	1.6	--	--
0.1	7.0	1.0	--	30.0	0.2	0.10	0.6	--	--	4.0	--	--
23.8	0.69	0.08	0.01	4.8	0.06	3.6	1.2	0.04	0.01	1.2	0.00092	6
0.2	10.0	2.0	0.01	80.0	0.4	0.2	0.8	--	--	1.0	--	--
25.4	1.7	0.83	0.09	6.8	0.42	4.2	6.8	0.07	0.67	0.85	0.00134	2
8.0	10.0	7.0	0.01	120.0	1.0	3.0	6.8	--	--	35.0	--	--
11.4	0.71	0.43	0.006	0.99	1.4	0.85	8.5	0.14	1.4	4.3	0.00076	5
0.1	10.0	3.0	--	80.0	0.2	0.10	1.0	--	--	4.0	--	--
1.8	0.74	0.03	0.37	27.6	0.09	0.92	4.6	--	0.37	--	0.0110	4
0.01	0.60	0.06	--	100.0	0.2	0.70	0.6	--	--	--	--	--
47.8	3.0	0.18	0.03	17.9	<0.06	2.4	6.0	<0.06	0.05	--	0.0017	7
0.09	6.0	2.0	--	10.0	0.1	0.1	0.6	--	--	--	--	--
8.5	1.7	0.07	0.02	3.4	0.06	1.7	25.6	0.03	1.7	2.6	0.0133	1
4.0	4.0	1.0	--	70.0	0.4	1.0	0.6	--	--	6.0	--	
4.3	1.2	0.14	0.01	8.6	<0.07	0.72	11.5	--	5.8	2.2	0.0007	3
0.01	3.0	1.0	--	1.0	0.1	0.1	0.6	--	--	8.0	--	--



1X 20634

a. Exposed Rods Prior to Removing Corrosion Products



1X 20634

b. Exposed Rods After Removing Corrosion Products

FIGURE 31. PHOTOGRAPH OF 7075-T6 ALUMINUM ROD SPECIMENS EXPOSED 42 DAYS IN SUMP WATER FROM LORING AFB, MAINE

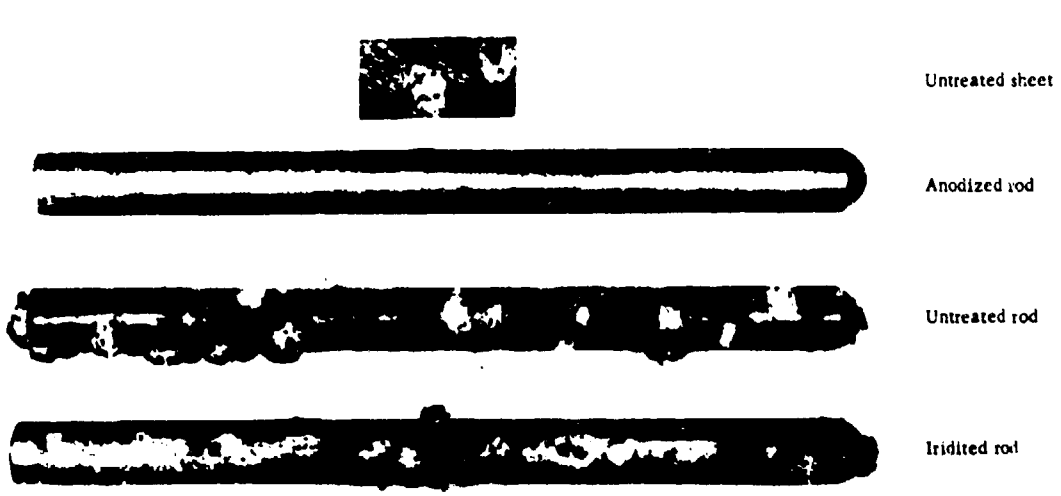
Note pits on untreated and iridited rods.

8X 20633

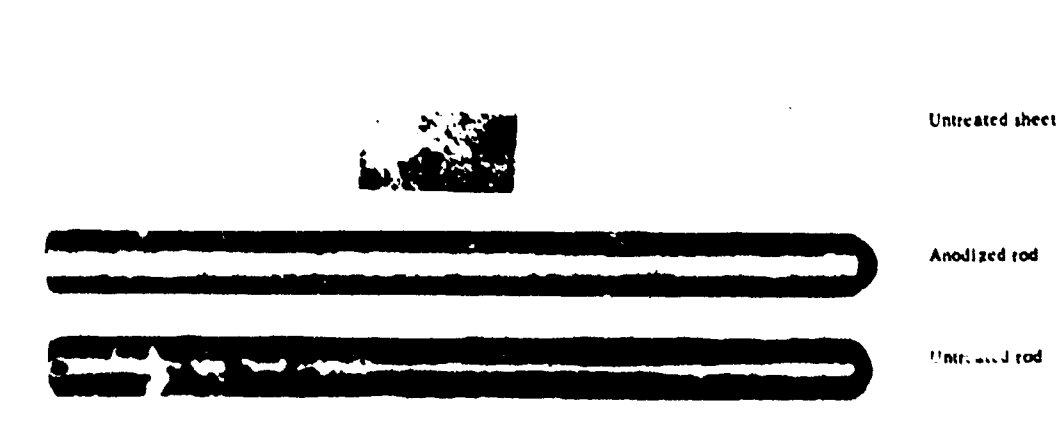
FIGURE 32. PHOTOGRAPH OF PITTED AREA ON INDITED 7075-T6 ALUMINUM ROD SPECIMEN EXPOSED 105 DAYS IN SUMP WATER FROM RAMEY AFB

75X 20635

FIGURE 33. CROSS SECTION THROUGH A PIT FROM INDITED 7075-T6 ALUMINUM ROD SPECIMEN EXPOSED 105 DAYS IN SUMP WATER FROM RAMEY AFB



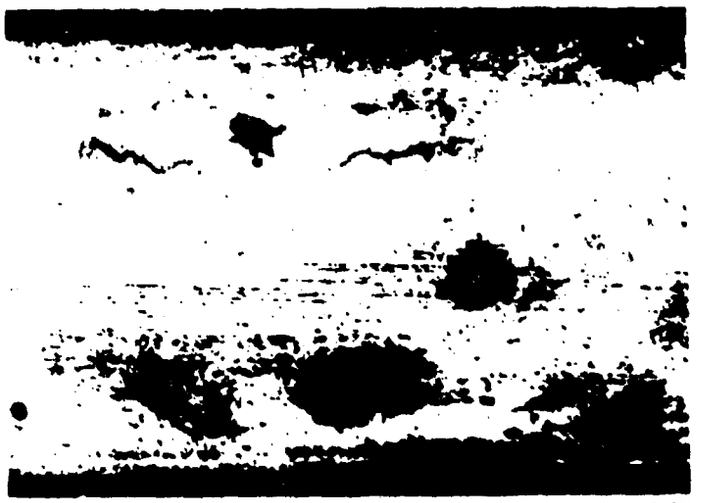
a. Exposed Rods Prior to Removing Corrosion Products



b. Exposed Rods After Removing Corrosion Products

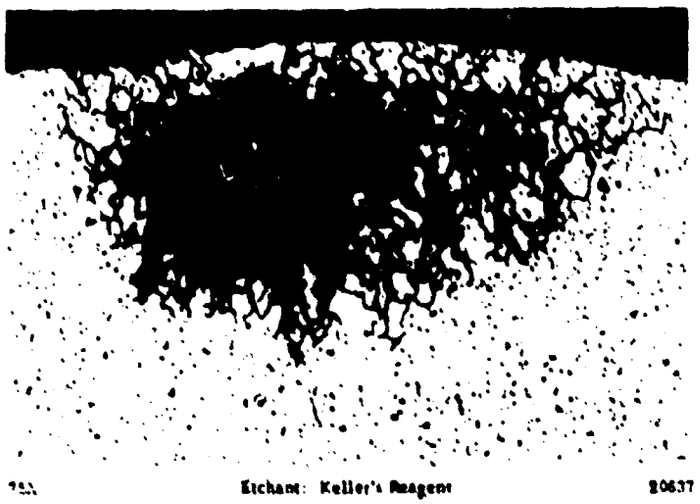
FIGURE 34. PHOTOGRAPH OF 7075-T6 ALUMINUM SPECIMENS EXPOSED 42 DAYS IN SUMP WATER FROM BERGSTROM AFB, TEXAS

Note blisters on iridited rod.



8X 20637

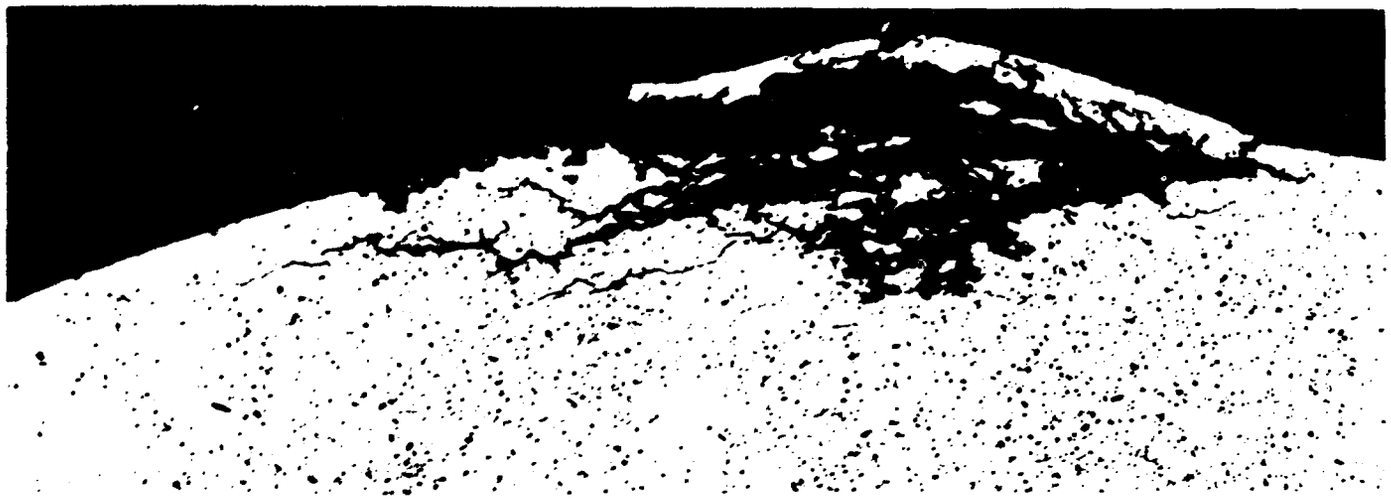
FIGURE 35. PHOTOGRAPH OF BLISTERS FOUND ON IRIDITED 7075-T6 ALUMINUM ROD SPECIMEN EXPOSED 42 DAYS IN SUMP WATER FROM BERGSTROM AFB, TEXAS



[illegible] Etchant: Keller's Reagent 20637

FIGURE 36. CROSS SECTION THROUGH BLISTERS FOUND ON IRIDITED 7075-T6 ALUMINUM ROD SPECIMEN EXPOSED 42 DAYS IN SUMP WATER FROM BERGSTROM AFB, TEXAS

Note intergranular attack.



75X Etchant: Keller's Reagent 20638

FIGURE 37. CROSS SECTION OF A BLISTER FOUND ON IRRADIATED 7075-T6 ALUMINUM ROD SPECIMEN EXPOSED 42 DAYS IN SUMP WATER FROM LEBGSTROM AFB, TEXAS

Note intergranular attack.

TABLE 29. ORDER OF CORROSIVITY OF SUMP SAMPLES TO IRIDITED 7075-T6 ALUMINUM (AFTER 42 DAYS OF EXPOSURE)

	General Corrosion Rate, mils/year		Calculated Maximum Pit Depth, mils/year
Biggs AFB	0.95	Bergstrom AFB	216.8
Bergstrom AFB	0.73	Homestead AFB	138.7
Homestead AFB	0.58	Biggs AFB	121.4
Loring AFB	0.56	Loring AFB	69.4
K. I. Sawyer AFB	0.18	Columbus AFB	43.3
Barksdale AFB	0.17	Barksdale AFB	34.7
Columbus AFB	0.08	K. I. Sawyer AFB	--

It will be noted that there is a slight difference in order of corrosivity of these various sump samples, depending upon which method of measurement is considered, i.e., general corrosion or annual pitting rate. Regardless of the method, the same four specimens remain at the top in each case. These are Biggs, Bergstrom, Homestead, and Loring Air Force Bases. However, it must be remembered that measurements of corrosivity are usually made on a statistical basis since such factors as slight differences in specimen edges or surfaces or the presence of occluded material can have a considerable effect on the results.

From the limited analytical data available on impurity content, it is difficult to draw complete conclusions regarding the effect of ion type and content on corrosion rate. On the other hand, it will be noted that, in general, the most corrosive sump samples are high in chlorides and heavy metal ions such as Cu, Fe, Ni, and Pb. Both chlorides and heavy metal ions are known to accelerate pitting attack of aluminum.

Effect of Sumps on Coated Aluminum Specimens. Studies were completed in which the corrosiveness of various water bottoms toward aluminum was determined. These water bottom or sump samples were obtained through RTD from aircraft at several SAC bases. It was requested that a study be made of the effect of these contaminated water-fuel mixtures on aluminum protected with standard Buna N and polyurethane coatings. This would provide information on the ability of these materials to prevent corrosion. In order to accelerate the possible effects of sump fluids, the immersed, coated specimens were placed in an oven at 140°F. After 14 days of this exposure, the samples were removed and examined. Results are summarized in Table 30. Damage to the coatings and corrosion of the aluminum varied somewhat. In general, the Buna N coatings were blistered while, with only two exceptions, the polyurethane coatings were not visibly affected. Figures 38 and 39 are colored photographs of the sump fluids and the coated rods, respectively, after exposure. It will be noted that the photograph of the sump fluids includes two samples not used in the rod exposure test. One of these is from Ramey AFB, and the other is a container filled with distilled water and used for color comparison. Photographs of the exposed specimens include only those coatings on Iridited aluminum since they were the most seriously affected.

TABLE 30. EFFECT OF AIRCRAFT SUMP COMPOSITES ON TOPCOATINGS

Exposure: 14 Days at 140°F[a]

Sump Source	Appearance of Coatings After Exposure	
	Two-Part Polyurethane Coating	Buna N Coating
Homestead AFB, Florida	No apparent effect	Slight leaching of color, numerous blisters.
Loring, AFB, Maine	No apparent effect	Leaching of color, blisters. Spots of white Al_2O_3 on all coatings[b]
Columbus AFB, Mississippi	No apparent effect	Small number of blisters. Al_2O_3 formation on Iridited rods[b]
Wright-Patterson AFB, Ohio	No apparent effect	Leaching of color, small number of blisters. Slight Al_2O_3 formation on irradiated rods[b]
Barksdale AFB, Louisiana	No apparent effect	Attached deposit of suspended matter on anodized rods. Al_2O_3 formation on Iridited rods.
K. I. Sawyer AFB, Michigan	Slight blistering on Iridited rods	Heavy deposit of suspended matter. Large blisters.
Biggs AFB, Texas	Deposit of dark suspended matter on coating. Very slight blistering on one Iridited rod	Deposited suspended matter. Al_2O_3 formation on all rods.[b]
Bergstrom AFB, Texas	Deposit of dark suspended matter on coating; otherwise, no apparent effect	Moderate blistering. Deposited suspended matter. Al_2O_3 formation (more severe on Iridited rods than on anodized rods).[b]

(a) Procedure: Coatings were given two applications on anodized and Iridited specimens of 3/8-inch 7075-T6 aluminum rods. Four specimens of each coating (2 anodized and 2 Iridited rods) were placed in 1-quart jars containing the sump samples. The jars were sealed and exposed in an air oven at 140°F for 14 days.

(b) Aluminum oxide (Al_2O_3) appeared as isolated mounds of white powder. Formation of Al_2O_3 indicates corrosion. Pits were found in the aluminum under each appearance of the white oxide growth.

FIGURE 38. COMPOSITE WATER BOTTOMS FROM AIRCRAFT FUEL SUMPS

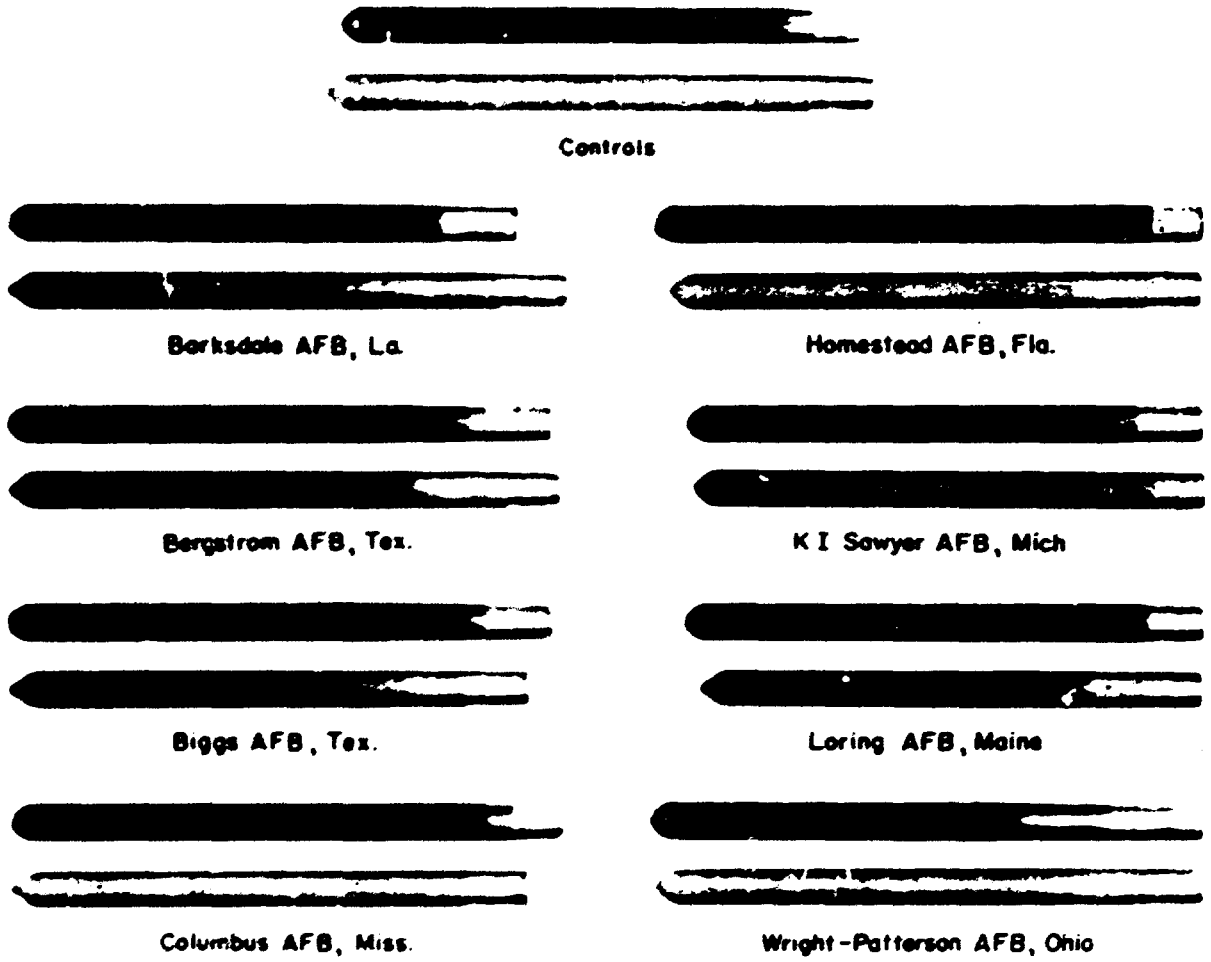


FIGURE 39. COATED IRIDITED RODS AFTER 2 WEEKS OF EXPOSURE AT 140 F TO SUMP FLUIDS

Dark color is Buna N Coating; lighter color is polyurethane coating.
Control samples exposed in distilled water.

Effect of Anti-Icer Content of Fuel Sumps on Corrosion

It has been suggested that the anti-icing additive which consists of 99.6 per cent ethylene glycol monomethyl ether and 0.4 per cent glycerine might act as a retardant for corrosion of aluminum as some polar materials do. An experiment was conducted to investigate this point. Strips and rod specimens of untreated, anodized, and Iridited 7075-T6 aluminum, some uncoated and others protected with Coating A or B, were exposed to Columbus tap water and to deionized water containing 20 or 30 per cent of the anti-icing additive. Results of this study are shown in Table 31. Photographs of the specimens are shown in Figure 40.

There was no evidence after 42 days of exposure at 80°F that the anti-icing additive inhibited corrosion. No corrosion was observed with or without the anti-icing

TABLE 31. EFFECT OF WATER TYPE AND PRESENCE OR ABSENCE OF ANTI-ICING COMPOUND ON CORROSION OF 7075-T6 ALUMINUM

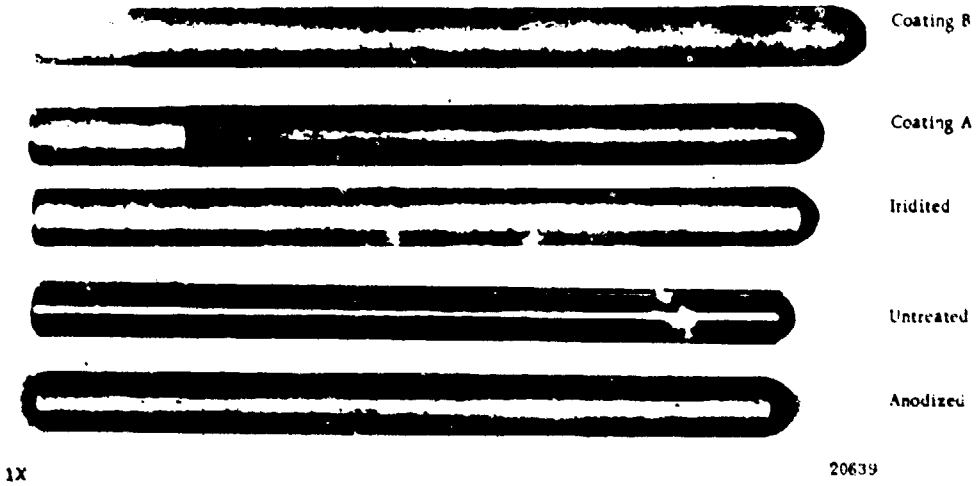
Exposure: 42 Days at 80° F

Solution	Specimen	General Corrosion Rate, mils/yr[a]	Calculated Max Pit Depth, mils/yr	Appearance	Max Pit Depth, mils
20%EGME[c]+0.2% glycerin + 79.8% deionized H_2O	Untreated sheet	0	--	No change, no pits	--
	Untreated rod	0.01	--	Two small areas of general corrosion, no pits	--
	Anodized rod	+0.02	--	No change, no pits	--
Ditto	Iridited rod	0.01	8.7	Localized attack, 2 small spots, 2 pits	1
-	Coating A rod	(b)	--	Coating has become lighter, no pits	--
-	Coating B rod	(b)	--	Ditto	--
30%EGME[c]+0.5% glycerin + 69.5% deionized H_2O	Untreated sheet	0	--	No change, no pits	--
	Untreated sheet	0.01	--	General corrosion, no pits	--
	Anodized rod	+0.03	--	No change, no pits	--
Ditto	Iridited rod	0.01	8.7	Seven areas of localized attack, 7 pits	1
-	Coating A rod	(b)	--	Much lighter coating, no pits	--
-	Coating B rod	(b)	--	Ditto	--
20%EGME[c]+0.2% glycerin + 79.8% tap water	Untreated sheet	0	4.3	No change, 1 pit	0.5
	Untreated rod	0	--	Very light general corrosion, no pits	--
	Anodized rod	+0.01	--	No change, no pits	--
Ditto	Iridited rod	0.1	30.4	One small speck localized attack, 1 pit	3.5
-	Coating A rod	(b)	--	Lighter color with spots of green, no pits	--
-	Coating B rod	(b)	--	Brighter appearance, no pits	--
Tap water	Uncoated sheet	0	--	Black film, no pits	--
Ditto	Untreated rod	0.04	--	Ditto	--
-	Anodized rod	+0.03	--	No change, no pits	--
-	Iridited rod	0	78.0	One area of localized attack, 1 pit	9
-	Coating A rod	(b)	--	Lighter color, with spots of green, no pits	--
-	Coating B rod	(b)	--	Brighter color, no pits	--

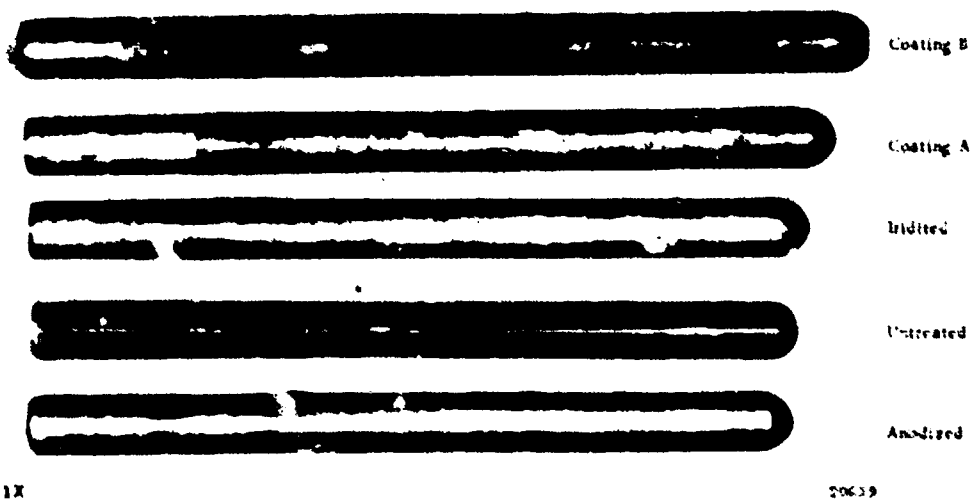
(a) "+" means rate based on weight gain $Al_2O_3 \cdot 3H_2O$; all others based on weight loss.

(b) No localized or other corrosion of aluminum was observed for rods coated with either Coating A or B. Weight loss of Coating A-coated rods was interpreted as a loss due to leaching out of polymer materials. Weight gain Coating B-coated rods was probably due to water uptake by the coating.

(c) EGME = ethylene glycol monomethyl ether.



a. 7075-T6 Aluminum Rods Exposed to 20.2 Per Cent Anti-icing Compound 79.8 Per Cent Deionized Water



b. 7075-T6 Aluminum Rods Exposed to Tap Water

FIGURE 40. PHOTOGRAPHS OF SPECIMENS EXPOSED 42 DAYS IN DEICING SOLUTIONS AND TAP WATER BEFORE REMOVING GROWTH

Anti-icing compound 20 per cent ethylene glycol monomethyl ether, 0.2 per cent glycerine.

additive on anodized specimens or on specimens coated with Organic Coatings A or B. The weight loss for Coating A probably represents material extracted from the coating. Weight gain for anodized rods represents the formation of $Al_2O_3 \cdot 3H_2O$. The same explanation may be true for weight gains of rods protected by Coating B, but this gain more than likely reflects water uptake by this coating.

In comparing the data in Table 31 with earlier data (see Tables 26 and 27), it will be noted that corrosion occurred on uncoated rods in the more recent experiments. There are a number of possible reasons for this, including such factors as age of the rod, differences in formed surfaces, possible residual contamination, and slight differences in alloy composition or crystal size. One other difference, of course, is the fact that water in the later tests contained anti-icer.

Synthetic Sump

As previously indicated, water sump samples from Air Force planes have corroded both uncoated and coated aluminum specimens. Also, there was a qualitative correlation between the heavy metal content of the sumps and their corrosiveness. An attempt was made to prepare a synthetic sump, the composition of which was based on the concentration of heavy metal ions found in the sump samples submitted by RTD (see Table 28). Since the chloride ion is known to accelerate corrosion of aluminum and the actual sump samples contained high concentrations of this ion, the synthetic sump was based entirely on chlorides of the various metals. Its actual composition is shown below:

	Salt		Metal Ion, ppm	Chloride, ppm
	Ppm	Weight, per cent		
$CaCl_2$	50	0.005	18	32
$CdCl_2$	1000	0.100	490	310
$MgCl_2$	50	0.005	6	18
NaCl	100	0.010	20	30
$ZnCl_2$	10	0.001	4.7	5.2
$CrCl_3 \cdot 6H_2O$	1	0.001	0.2	0.3
$CuCl_2 \cdot 2H_2O$	1	0.001	0.4	0.4
$FeCl_3$	5	0.005	1.7	3.1
$MnCl_2 \cdot 4H_2O$	5	0.005	1.4	1.8
$NiCl_2 \cdot 6H_2O$	1	0.001	0.2	0.3
$PbCl_2$	1	0.001	0.7	0.3
Total	1224	0.135	543.3	461.6

Aluminum rods coated with a polyurethane and a Buna N coating were exposed to the artificial sump for 2 weeks at 140° F. Corrosion of the metal occurred in about the same length of time as that observed for actual sumps, although the patterns were somewhat different. In the synthetic sump, some corrosion of anodized aluminum coated with the polyurethane coating took place, whereas anodized aluminum coated with the polyurethane did not corrode in the natural sumps. In subsequent 42-day exposures of untreated, anodized, and Iridited 7075-T6 aluminum, the corrosion data (Table 24) do not entirely agree with those obtained in actual sumps. For example, the Iridited aluminum

consistently showed a fairly high degree of corrosion in actual sumps, while little or no corrosion of the same surface was evident in the synthetic sump. Also, untreated aluminum, which showed a weight loss in sumps obtained in the field, showed a consistent weight gain in the synthetic system. This is certainly not wholly unexpected in what amounts to an isolated experiment.

Neither the analysis of sumps nor the study of corrosion was a primary objective of this study. However, a limited amount of corrosion work was done at the request of RTD because of its importance to the program and its connection with Battelle's study of coatings. Results of this single attempt at devising a synthetic sump were sufficiently interesting to indicate possible utility of such an approach in screening of coatings, studying aluminum treatments, and possible further elucidation of the role of microorganisms in corrosion.

It should be pointed out, however, that a number of additional factors should be taken into consideration in devising a synthetic corrosion medium. For example, in this single experiment, no anti-icing additive was present, whereas actual sumps contain up to 35 per cent additive. This may explain why blistering observed on coatings in real sumps did not occur in the laboratory-prepared medium. Further, it is known from reports of work conducted on synthetic sumps by another contractor that considerable time has been spent on studying the effect of various other ions including nitrates, sulfates, etc. These were not present in the synthetic sump prepared here. Thus, it is apparent that more work would be necessary to define an ideal composition. Such a composition cannot be based entirely on an analysis of field samples since these can themselves vary considerably while exhibiting similarities in corrosivity. Finally, corrosion can be studied best on a statistical basis. Thus, a much larger experimental program would be needed to obtain conclusive results.

APPENDIX

LABORATORY MEDIA AND METHODS

Laboratory Media

Bushnell-Haas Mineral Salts Medium

Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Calcium chloride ($CaCl_2$)	0.02 g
Potassium phosphate, monobasic (KH_2PO_4)	1.0 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Ammonium nitrate (NH_4NO_3)	1.0 g
Ferric chloride ($FeCl_3$) (15 g per 25 ml distilled water)	2 drops
Distilled water	1000 ml

pH adjusted 7.0-7.2 with dilute sodium hydroxide (NaOH). Twenty grams of agar was added to the above formula when an agar medium was needed. Five grams of glucose was added to the formula in studies where a simple carbon source was required.

Modified Bushnell-Haas Mineral Salts Medium

The above-described formulation was modified by deleting $FeCl_3$, and adding Arnon's Micrometabolic Element Solution, which was originally described by Hoagland and Arnon* and further modified by Battelle. The latter modification consisted of substituting Sequestrine 330 iron chelate (Geigy Chemical Company) for ferric tartrate. Sequestrine 330 is sodium ferric diethylene triamine penta-acetate (10 per cent iron). The solution used in these studies contained:

H_3BO_3	2.5 g
$MnCl_2 \cdot 4H_2O$	1.5 g
$ZnCl_2$	0.1 g
$CuCl_2 \cdot 2H_2O$	0.05 g
MoO_3	0.05 g
Distilled water	1000 ml

*Hoagland, D. R., and Arnon, D. I., University of California Agriculture Experiment Station Circular No. 347 (1938).

One ml of this micrometabolic solution and 1 ml of the diluted iron chelate (1.8 g Sequestrine 330 diluted with 100 ml distilled water) were added to 1000 ml of the Bushnell-Haas mineral salts medium (minus $FeCl_3$) to form the modified version.

Bacto Nutrient Agar (Difco)

Bacto-beef extract	3 g
Bacto-peptone	5 g
Bacto-agar	15 g
Distilled water	1000 ml

Final pH = 6.8

Bacto Tryptone Glucose Extract Agar (Difco)
(For Jet-Fuel Isolates)

Bacto-beef extract	3 g
Bacto-tryptone	5 g
Bacto-dextrose (d-glucose)	1 g
Bacto-agar	15 g
Distilled water	1000 ml

This medium was supplemented with 0.1 per cent bacto-yeast extract when needed.

Final pH = 7.0

Bacto Thioglycollate Medium (Difco)
(For Clostridium sporogenes)

Bacto-yeast extract	5 g
Bacto-casitone	15 g
Sodium chloride	0.5 g
l-Cystine	0.25 g
Thioglycollic acid	0.3 g
Bacto-agar	0.75 g

Distilled water	1000 ml
Final pH = 7.2	

API Sulfate-Reducing Broth
(For Desulfovibrio desulfuricans)

Sodium lactate, USP	4.0 ml
Yeast extract	1.0 g
Ascorbic acid	0.1 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Dipotassium phosphate (K_2HPO_4-anhydrous)	0.01 g
Ferrous ammonium sulfate $[Fe(SO_4)_2(NH_4)_2 \cdot 6H_2O]$	0.1 g
Sodium chloride (NaCl)	10.0 g
Distilled water	1000 ml
Final pH = 7.5	

Trypticase Soy Agar
(For Sphaerotilus natans)

Trypticase	15 g
Phytone	5 g
Sodium Chloride	5 g
Agar	15 g
Distilled water	1000 ml
Final pH = 7.3.	

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13. ABSTRACT

This program was part of a major study by the Air Force to overcome problems created by the undesirable growth of microorganisms in aircraft jet-fuel tanks and ground storage tanks. Problems attributed to microbial growth include plugging of fuel gauges, destruction of topcoatings, and corrosion of metal parts.

It was shown that certain species of bacteria and fungi, isolated from JP-4 fuel, grow rapidly on coatings in laboratory exposures. Damage to coatings after one year of exposure was minimal, suggesting that growth alone is not as important as growth augmented by other factors such as corrosive materials in fuel-tank water bottoms. For example, aircraft fuel sump samples (without microorganisms) from various USAF bases were shown to be corrosive to both uncoated and coated 7075-T6 aluminum and to cause blistering of a topcoating.

Although coating damage and metal corrosion could not be attributed to microbial action alone, control of microbial growth in fuel tanks is still desirable. Several biocides were found which inhibit growth when added to coatings. Two of these in particular were effective at relatively low concentrations and are recommended for further study. It was also shown that three biocides selected for addition to fuel are not harmful to most existing fuel-system coatings and elastomeric components, although one caused embrittlement of a nitrile rubber specimen.

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